Immunohistochemical Evaluation of Estrogen Receptor (ER) and Progesterone Receptor (PR) in Endometrial Carcinomas and Its Precursors

A DISSERTATION SUBMITTED IN PART FULFILMENT OF THE REGULATION

FOR THE AWARD OF THE DEGREE OF M.D. PATHOLOGY BRANCH III.

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A dissertation in part fulfillment of the rules and regulations for the M.D. Branch III (Pathology) Degree Examination of the Tamil Nadu Dr. M.G.R Medical University, to be held in May 2019

CERTIFICATE

This is to certify that this dissertation titled **"Immunohistochemical Evaluation of Estrogen Receptor (ER) and Progesterone Receptor (PR) in Endometrial Carcinomas and Its Precursors"** is a bonafide work done in the department of General Pathology by Dr. Rima S (Postgraduate Registrar), in part fulfillment of the rules and regulations for the M.D. Branch III (Pathology) Degree Examination of the Tamil Nadu Dr. M.G.R Medical University, to be held in May 2019.

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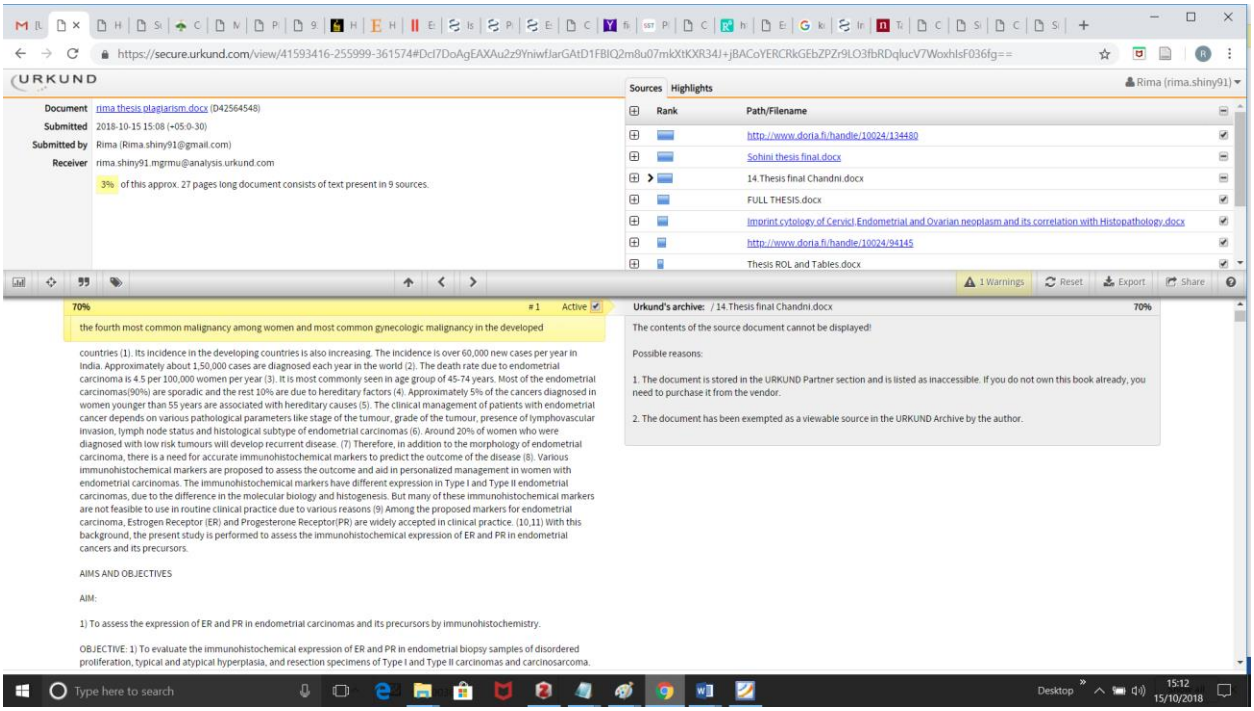

This is to certify that this is the dissertation work entitled “Immunohistochemical evaluation of Estrogen receptor (ER) and Progesterone Receptor (PR) in Endometrial carcinomas and its precursors” of the candidate Rima. S with registration Number 201613355 for the award of Degree of MD Pathology in the Branch III. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows three percent (3%) of plagiarism in the dissertation.

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- **Dr. RIMA. S**

Abbreviations

ER	- Estrogen Receptor
PR	- Progesterone Receptor
DPEM	- Disordered proliferation
ET	- Endometrial thickness
SEIC	- Serous Endometrial Intraepithelial Carcinoma
FIGO	- International Federation of Gynecology and Obstetrics
BMI	- Body Mass Index

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INTRODUCTION:

Endometrial carcinoma is the fourth most common malignancy among women and most common gynecologic malignancy in the developed countries (1). Its incidence in the developing countries is also increasing. The incidence is over 60,000 new cases per year in India. Approximately about 1,50,000 cases are diagnosed each year in the world (2). The death rate due to endometrial carcinoma is about 4.5 per 100,000 women per year (3). It is most commonly seen in age group of 45-74 years.

Most of the endometrial carcinomas (90%) are sporadic and the rest 10% are due to hereditary factors (4). Approximately 5% of the cancers diagnosed in women younger than 55 years are associated with hereditary causes (5). The clinical management of patients with endometrial cancer depends on various pathological parameters like stage of the tumour, grade of the tumour, presence of lymphovascular invasion, lymph node status and histological subtype of endometrial carcinomas (6). Around 20% of women who were diagnosed with low risk tumours will develop recurrent disease. (7)

Therefore, in addition to the morphology of endometrial carcinoma, there is a need for accurate immunohistochemical markers to predict the outcome of the disease (8). Various immunohistochemical markers are proposed to assess the outcome and aid in personalized management of endometrial carcinomas. These immunohistochemical markers have different expression in Type I and Type II endometrial carcinomas, due to the difference in the molecular biology and histogenesis. But many of these immunohistochemical markers are not feasible to use in routine

clinical practice due to various reasons (9)

Among the proposed markers for endometrial carcinoma, Estrogen Receptor(ER) and Progesterone Receptor(PR) are widely accepted in clinical practice (10,11). With this background, the present study is performed to assess the immunohistochemical expression of ER and PR in endometrial cancers and its precursors.

AIMS AND OBJECTIVES:

AIM:

1) To assess the expression of ER and PR in endometrial carcinomas and its precursors by immunohistochemistry.

OBJECTIVE:

1) To evaluate the immunohistochemical expression of ER and PR in endometrial biopsy samples of disordered proliferation, typical and atypical hyperplasia, and resection specimens of Type I and Type II carcinomas and carcinosarcoma.
2) To evaluate the immunohistochemical expression of ER and PR in resection specimens of Type I, Type II endometrial carcinomas, carcinosarcomas and correlate it with histological features such as lymphovascular invasion, lymph node status, grade and stage of tumour.

REVIEW OF LITERATURE:

Epidemiology:

Endometrial carcinoma is the third most common female genital tract malignancy in females of south east Asia (12). Though, endometrial carcinoma is more common in developed countries, the annual incidence in developing countries is projected to increase because of change in life style and high incidence of obesity (13). They usually arise as a continuum of premalignant lesions such as disordered proliferation, typical hyperplasia and atypical hyperplasia. Endometrial carcinomas are broadly classified as Type I (70-80%) and Type II (10-20%) carcinomas. Type I carcinomas follow estrogen dependent pathway, develop in a background of hyperplastic endometrium and are usually low grade. Type II carcinomas follow estrogen unrelated pathway, develop in an atrophic endometrium and are high grade. (2)

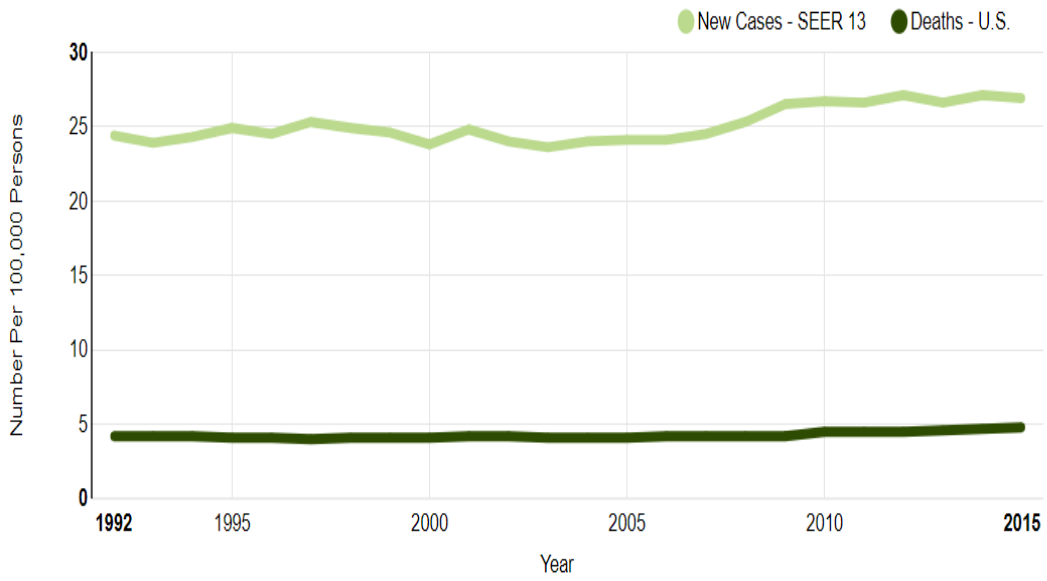

Figure 1: Incidence of endometrial carcinoma over a period of 15 years (3)

Histologically the endometrium is made of two layers (14)

1. Stratum basale

2. Stratum functionalis.

The cyclical changes occurring in the endometrium are mostly controlled by estrogen and progesterone (15). The functionalis layer occupies almost one third of the endometrium and is responsible for proliferation and tissue degeneration. The stratum basalis lies beneath the stratum functionalis and has regenerative function. (16)

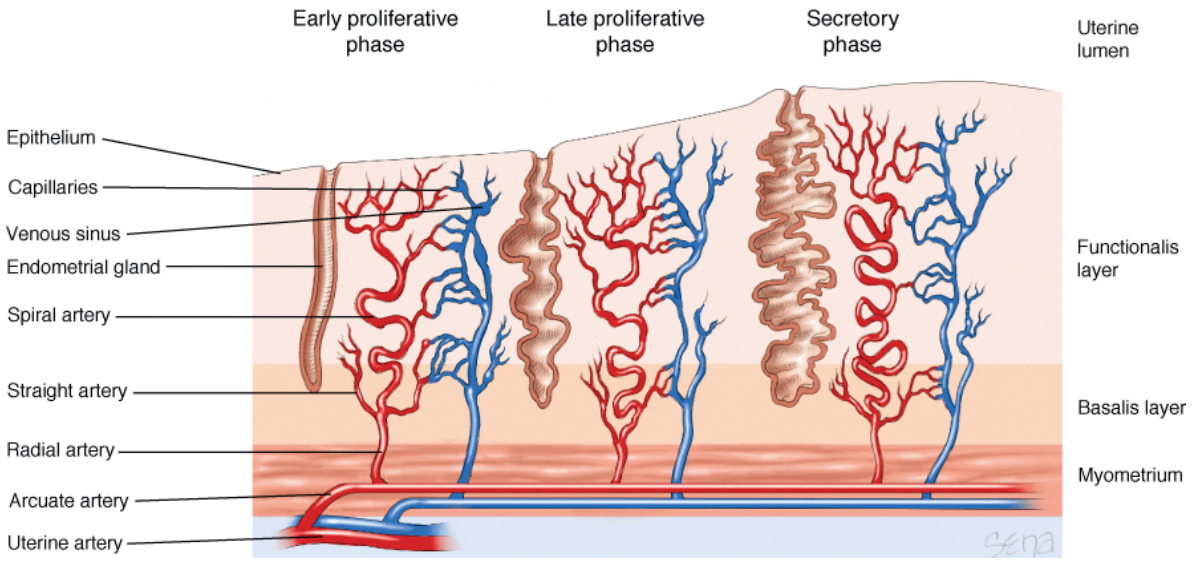

Figure 2: Phases of menstrual cycle (20)

The morphological changes over the entire menstrual cycle are tightly controlled by ovarian steroid hormones (estrogen and progesterone). In 1970 and 1980, using auto radiographic techniques, the presence and the binding capacities of estrogen receptor(ER) and progesterone receptor (PR) across the menstrual cycle were studied (17).

Estrogen receptors:

Estrogen receptor is one of the important members of steroid hormone super family of nuclear receptors (18). It is an important sex hormone for the development and physiological functions of reproductive organs. Estrogen also plays a main role in metabolism, bone remodeling and functioning of the cardiovascular system (19). The biological activity of estrogen is mediated by its binding to the estrogen receptors. There are two types of estrogen receptor.

1. Estrogen receptor Alpha (ERα)
2. Estrogen receptor Beta (ERβ)

The estrogen receptor alpha (ERα) is located on the chromosome 6. The estrogen receptor beta (ERβ) is located on the chromosome 14 (20). The physiologic and biological activity of estrogen is mainly dependent on the distribution of the receptors in the target organs. Estrogen receptor alpha (ERα) is mainly seen in uterine parenchyma, stroma of prostate, Leydig cells, theca cells of ovary, liver and breast. On the other hand, ERβ is expressed mainly in prostatic epithelium, granulosa cells of ovary, bone marrow and brain (21).

The estrogen receptor has five domains with different functions. (22)

1. A/B Domain- Contains the activator function 1 (AF1) responsible for the transcriptional activity.
2. C Domain- DNA binding domain.
3. D domain- hinge region

4. E/F receptor- Interaction site and has ligand dependent activator function 2 (AF2).

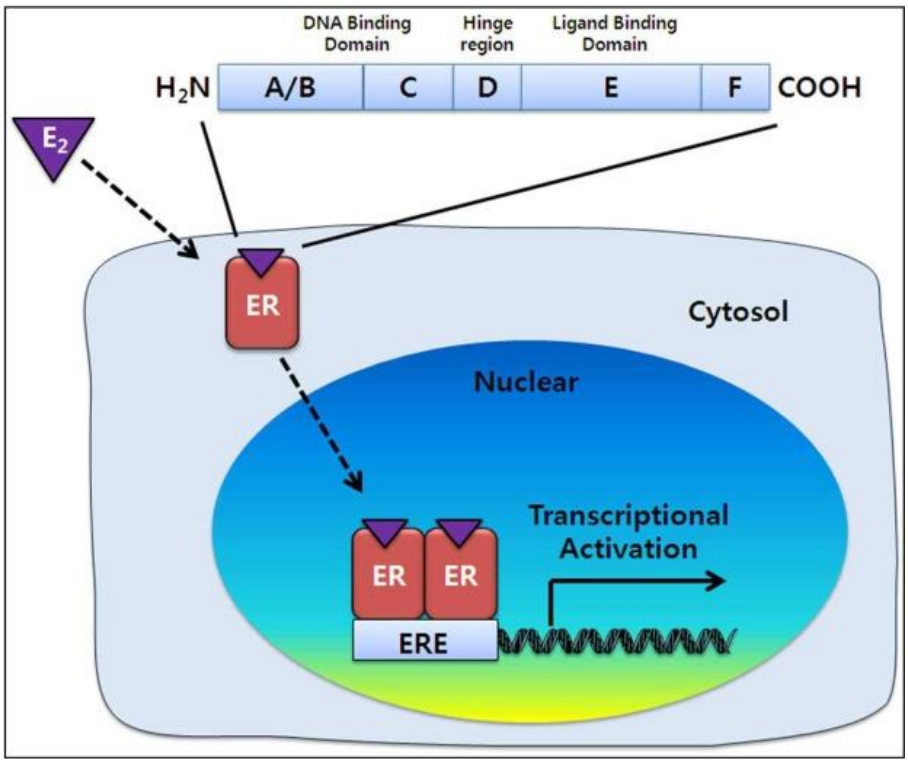

Figure 3: Mechanism of estrogen receptor signaling pathway (26)

The estrogen receptors remain in an inactive form, due to the presence of Heat Shock Protein (in the absence of hormones).

Role of estrogen receptors in the endometrium:

ERα and ERβ are expressed on the epithelial and stromal cells of the endometrium. According to Matsuzaki et al, the expression of ERβ is much lower than that of ERα expression throughout the menstrual cycle (23). Though the expression of ERβ is low, it is expressed in epithelial, stromal and endothelial cells of the endometrium. Among these, the expression of ERβ is relatively high in the epithelial cells.

ERα is highest in the estrogen dominant proliferative phase of the menstrual cycle. Expression of both the estrogen receptors (ERα and ERβ) are high in the early proliferative endometrium, mid proliferative endometrium and early secretory endometrium. Expression of both the estrogen receptors subsequently decreases in the mid secretory endometrium and late secretory endometrium (24). In the late secretory phase, ERβ is expressed more than ERα in the endometrial stroma. This is due to the fact that the expression of both the estrogen receptors in the epithelium decreases as the cycle enters the secretory phase, but the stromal expression of ERβ is relatively maintained or increased (25).

ER expression in post-menopausal status:

The hormonal environment of pre and post-menopausal endometrium is entirely different. In postmenopausal women, the endometrium is subjected to low levels of estradiol and absent progesterone (26). The expression of ERβ in post-menopausal endometrium is weaker than that of ERα in both the epithelial and stromal elements. Zang et al proposed that the expression of ERβ increases in the post-menopausal endometrium, when the patients are given combined treatment with E2 and testosterone (27).

Progesterone hormone and its receptors:

Progesterone plays an important role in the female reproductive system. In combination with estrogen (estradiol), it helps in the implantation and maintenance of pregnancy (28). The progesterone exerts most of its actions in the following tissues.

1. Epithelial cells in the endometrium.
2. Stromal cells in the endometrium.
3. Stromal fibroblasts.
4. Smooth muscle cells in the myometrium.
5. Glandular epithelial cells in the cervix.

The actions of progesterone in the female reproductive tract are controlled and mediated by progesterone receptors. There are two types of receptors based on its location in the cell (29).

1. Nuclear Progesterone receptor- Ligand activated and involved in genomic actions.
2. Cytoplasmic Progesterone receptor- G- Protein coupled receptor and single trans membrane receptors. These receptors are involved in non-genomic actions.

The progesterone receptor gene is located on the chromosome 11. There are two isoforms of progesterone receptors. The activities of progesterone are mediated by the combined effects of these isoforms.

1. Progesterone Receptor A- PRA
2. Progesterone Receptor B-PRB.

The progesterone receptor is composed of four regions.

1. N-Terminal domain- Activation and Inhibitory functions.
2. DNA binding domain (DBD).
3. Hinge region
4. Ligand binding domain (LBD)

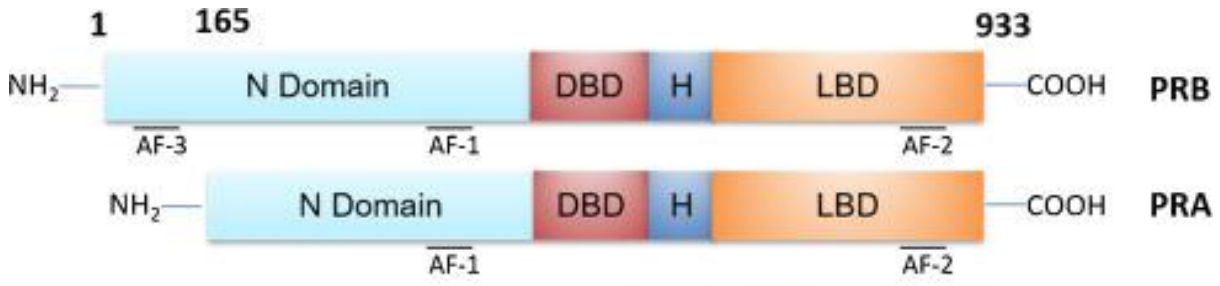

Figure 4: Structure of progesterone receptor (29)

CLASSIFICATION:

In 1983, Bokhman, first classified endometrial carcinoma based on clinico-pathological features and molecular background into two groups:

1. Type I
2. Type II

Endometrioid carcinoma is the most common histological variant of Type I carcinomas. Serous and clear cell carcinoma are the most common histological variant of Type II carcinomas. But there are few histological subtypes(example: carcinosarcoma) in which

definite categorization cannot be made (4). Based on this classification a ‘dualistic model of endometrial carcinogenesis’ was proposed (4).

Dualistic model of endometrial carcinogenesis:

According to the ‘dualistic model of endometrial carcinogenesis’, it is speculated that endometrial carcinoma (Type I) develops following a continuum of precursor lesions ranging from disordered proliferation, typical and atypical hyperplasia and Type II carcinomas develop from endometrial intraepithelial neoplasia (30). Hence the exact diagnosis of precursor lesions in routine endometrial biopsies is very crucial in patient care. The precursor lesions are diagnosed in endometrial biopsies by histo-morphological features such as presence or absence of nuclear atypia, which has poor reproducibility and high inter-observer variability (14). Hence the use of immunohistochemical stains and mutational analysis can aid in the diagnosis of premalignant lesions and endometrial carcinoma in routine endometrial biopsies.

	Type I	Type II
Associated clinical features	Metabolic syndrome: obesity, hyperlipidaemia, hyperglycaemia, and increased oestrogen concentrations	None
Grade	Low	High
Hormone receptor expression	Positive	Negative
Histology	Endometrioid	Non-endometrioid (serous, clear-cell carcinoma)
Genomic stability	Diploid, frequent microsatellite instability (40%)	Aneuploid
TP53 mutation	No	Yes
Prognosis	Good (overall survival 85% at 5 years)	Poor (overall survival 55% at 5 years)

Table 1: Dualistic classification of endometrial cancer (31)

I.EPITHELIAL TUMOURS AND PRECURSORS:

Precursors:

Typical hyperplasia.

Atypical hyperplasia/Endometrioid intraepithelial neoplasia.

Endometrioid carcinoma

Squamous differentiation

Villoglandular

Secretory

Mucinous carcinoma

Serous endometrial intraepithelial carcinoma.

Clear cell carcinoma

Neuroendocrine tumours

II.MESENCHYMAL TUMOURS

Leiomyoma

Smooth muscle tumour of uncertain malignant potential

Leiomyosarcoma

Endometrial stromal and related tumours.

Miscellaneous

III.MIXED EPITHELIAL AND MESENCHYMAL TUMOURS

Adenomyoma

Atypical polypoid adenomyoma

Adenofibroma Adenosarcoma Carcinosarcoma
IV.MISCELLANEOUS TUMOURS
V.LYMPHOID AND MYELOID TUMOURS
VI.SECONDARY TUMOURS

Table 2: The WHO classification of tumours of uterine corpus

TYPE I ENDOMETRIAL CARCINOMA:

Type I endometrial carcinoma accounts for approximately 70-80% of total sporadic cases of endometrial carcinoma (4). Type I endometrial carcinoma is more common than Type II (2) and usually have indolent clinical behavior as compared to Type II tumours.

Risk factors:

Estrogen excess:

Type I endometrial carcinomas are most commonly associated with unopposed estrogen action (32). The relationship between estrogen excess and the development of endometrial cancer is explained by 'unopposed estrogen hypothesis' (32). According to the hypothesis, the development of endometrial carcinoma is associated with high levels of estrogen and low levels of progesterone, and henceforth the effects of estrogen (such as inducing mitosis and cell division) are not counterbalanced by progesterone

(33). In post-menopausal women the baseline levels of circulating sex hormones is low. On treatment with estrogen only hormone replacement therapy (HRT) there is a two to three times increase in the risk of developing endometrial carcinoma. The use of combined hormone replacement therapy and oral contraceptive are associated with a protective effect (34). The other constitutional factors are early age of menarche and late age of menopause.

b) Nulliparity:

Nulliparity is associated with increased risk (up to three fold) due to chronic anovulatory cycles, which in turn result in unopposed estrogenic stimulation. Hence the development of endometrial carcinoma is more in married nulliparous women than for unmarried women (35).

c) Obesity:

Obesity is associated with approximately 40% of endometrial carcinomas (36). In obese individuals there is extra glandular estrone formation due to peripheral conversion of androgens in the adipose tissue as well as insulin resistance, which results in decreased production of sex hormone binding globulin in the liver and leads to excess circulating estrogen (37). A Body Mass Index (BMI) more than 25 kg/m^2 increases the risk of developing endometrial carcinoma by two fold (38).

d) Life style factors:

The development of endometrial cancer is associated with the total calorie and protein intake (2). It has been speculated that a diet which is rich in vegetables and

fruits, reduced amounts of saturated fatty acids and moderate intake of wine is associated with decreased incidence of endometrial cancer (39).

e) Others:

Diabetes mellitus, Tamoxifen therapy, polymorphisms in the estrogen receptor are also associated with increased incidence of endometrial carcinoma (2).

Genetic factors:

A number of cancer causing genes have been identified in the development of endometrial carcinoma (Type I) such as PTEN, PIK3CA, PI3K, microsatellite instability and K-ras mutation. Among the various genetic alterations, PTEN (phosphatase and tensin homologue) gene is the most frequently mutated gene in Type I endometrial carcinoma(30-54%) (40).

Genetic Alteration	Type 1 Carcinoma (%)	Type 2 Carcinoma (%)
PTEN inactivation	50–80	10
K-ras mutation	15–30	0–5
β-catenin mutation	20–40	0–3
Microsatellite instability	20–40	0–5
p53 mutation	10–20	80–90
HER-2/neu	10–30	40–80
p16 inactivation	10	40
E-cadherin	10–20	60–90

Table 3: Genetic alterations in Type 1 and Type II endometrial carcinomas (4)

Pathogenesis of Type I endometrial carcinomas:

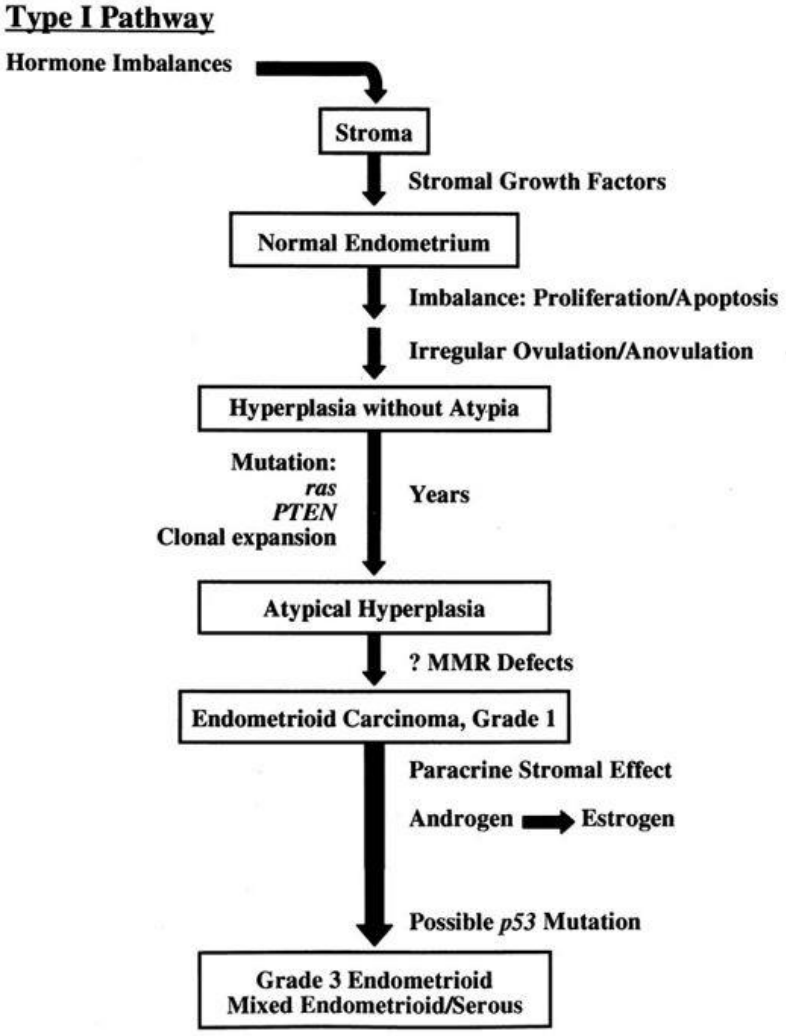

Figure 5: Pathogenesis of Type I pathway (41)

Type I endometrial carcinomas, represent estrogen related carcinomas and they develop from a background of premalignant lesion such as typical hyperplasia and atypical hyperplasia (42). The World Health Organization (WHO) proposed a four tier system for the classification of endometrial hyperplasia in 1994. The term glandular

hyperplasia was first introduced by Cullen in 1900 (43). This classification is based on cytologic and architectural abnormalities (2).

Typical hyperplasia 1. Simple hyperplasia without atypia 2. Complex hyperplasia without atypia. (adenomatous without atypia)
Atypical hyperplasia 1. Simple atypical hyperplasia 2. Complex atypical hyperplasia (adenomatous with atypia)

Table 4: WHO classification of precursor lesions of endometrium (1994)

These categories are descriptive in nature and the interpretation is subjective. These individual categories do not specify individual clinical management algorithms (44). Most of the studies, proposed that the risk of progression of hyperplasia to carcinoma depends more on the cytological atypia, than architectural atypia (45). According to Lacey et al, the risk of progression of hyperplasia without atypia to carcinoma was only 10%, on the other hand the risk of progression of hyperplasia with atypia to carcinoma was 40% (46). Following this, the WHO simplified the classification in 2014 as a two tier system.

1. Typical hyperplasia
2. Atypical hyperplasia / Endometrioid intraepithelial neoplasia.

Typical hyperplasia occurs secondary to unopposed estrogen stimulation (47).

The definition is “Exaggerated proliferation of glands of irregular size and shape, with an associated increase in the gland to stroma ratio compared with proliferative endometrium, but without significant cytological atypia” (48).

The development of the lesion is strongly associated with unopposed estrogenic stimulation, and hence the duration and dose of estrogen exposure affects the overall histological picture. According to Kurman et al, the risk of progression to carcinoma occurs in 1-3% of patients with hyperplasia without atypia (45). The term disordered proliferation is used when there is proliferation of glands with no cytological atypia, that exceeds that of proliferative endometrium but falls short of the crowding in hyperplasia (48).

Atypical hyperplasia/Endometrioid intraepithelial neoplasia:

Endogenous or exogenous hyperestrinism is the important risk factor for the development of atypical hyperplasia (49). Cytological atypia superimposed on endometrial hyperplasia defines atypical hyperplasia (48). The distinction between typical hyperplasia and atypical hyperplasia is based on cytological atypia (following nuclear features) (45,50).

1. Nuclear enlargement.
2. Nuclear pleomorphism.
3. Rounding of nucleus.
4. Loss of polarity.
5. Presence of nucleoli.

These histological features are subjective with much intra-observer and Inter- observer variability. In approximately 25-40% of women, atypical hyperplasia coexist with carcinoma (51). Atypical hyperplasia shows most of the genetic changes that are seen in Type I endometrial carcinomas, such as microsatellite instability, PAX2 inactivation and PTEN mutation (52). The risk of progression of atypical hyperplasia to carcinoma is 25-33% during the first year of follow up (45,51,53)

Management of preneoplastic lesions:

As the risk of progression of atypical hyperplasia is around 30%, surgery is the recommended treatment option. But depending on the fertility status and associated comorbidities treatment with progestins can be considered.

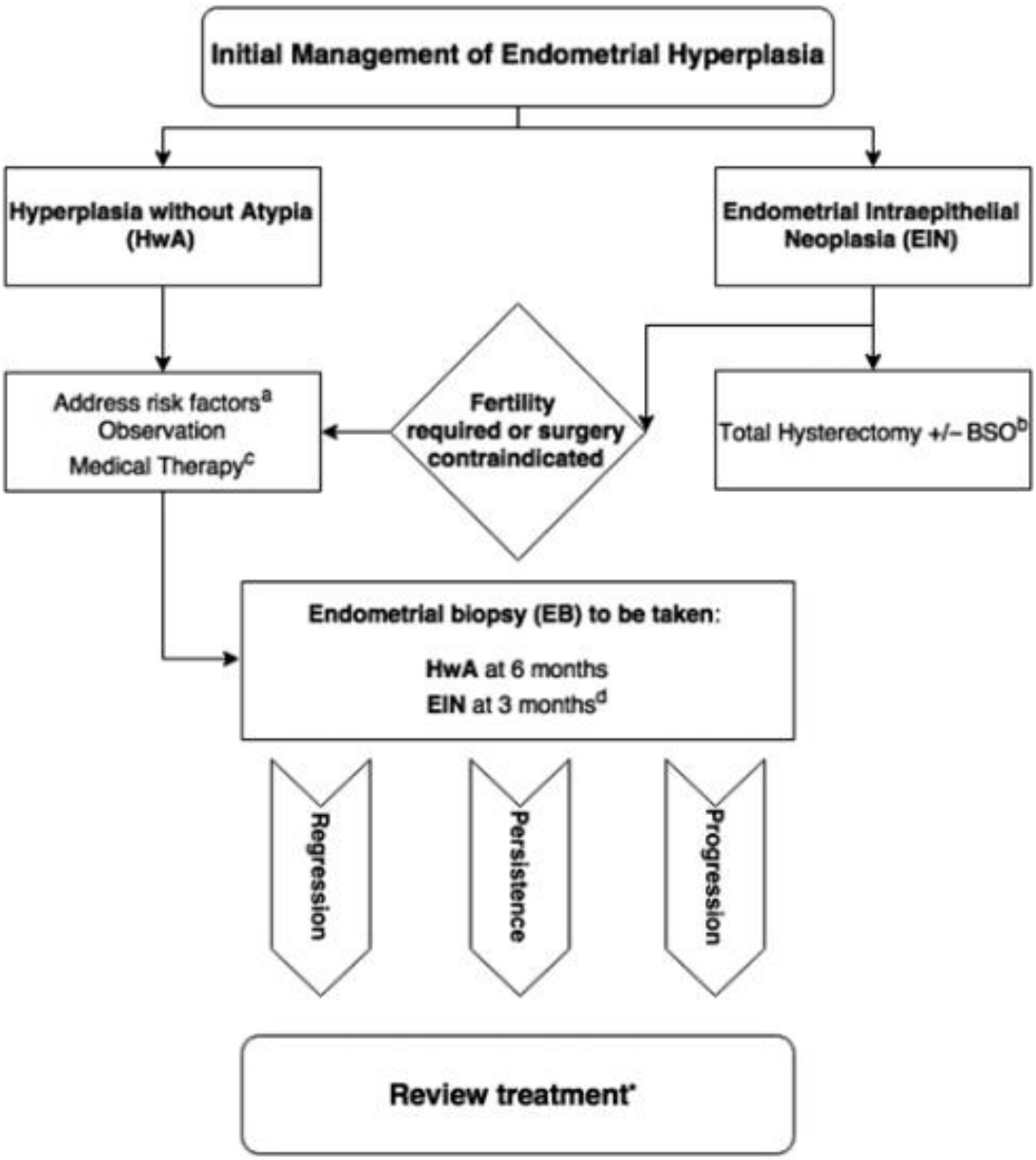

Figure 6: Management of endometrial precursor lesions (14)

Type I endometrial carcinomas:

Endometrioid adenocarcinoma is the prototype of Type I endometrial carcinomas (1). Postmenopausal women with high estrogen levels, patients with polycystic ovarian syndrome, menarche at an early age, nulliparity and patients with estrogen

producing ovarian tumours are at risk for developing endometrial carcinoma (54). Patients with Lynch Syndrome and Cowden syndrome are also at an increased risk of developing endometrial carcinoma (55). The average age of diagnosis is around 63 years (56). The most common presenting symptom is post-menopausal bleeding. Postmenopausal bleeding is defined as uterine bleeding after permanent cessation of menstruation resulting from loss of ovarian follicular activity (57). The other symptoms include vaginal discharge and pelvic pain. In few cases, abnormal cells may be found in routine cervical cytology (PAP smear) (58). The initial imaging done in patients with abnormal uterine bleeding is pelvic ultrasonography. A cut off value >11mm endometrial thickness is associated with an increased risk of cancer by 6.7% (59).

Gross appearance:

Most of the endometrial tumours have an exophytic diffuse growth. A few may present as tan nodules in the endometrium. Hemorrhage and necrosis are common.

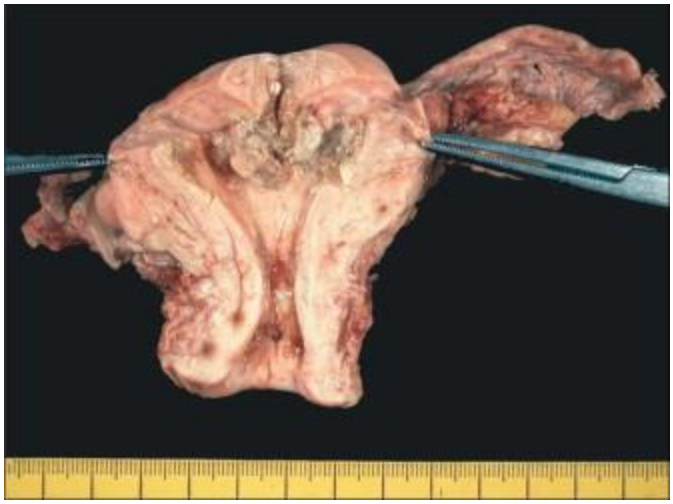

Figure 7: Gross appearance of endometrioid carcinoma (41)

Histology:

Well differentiated endometrioid carcinoma is differentiated from atypical hyperplasia based on the following features (60).

1. An irregular infiltration of glands with desmoplastic response.
2. Confluent glandular and cribriform pattern in which glands are uninterrupted by the stroma.
3. Excessive papillary pattern(villoglandular)
4. Stromal replacement by masses of squamous epithelium.

Patterns in endometrioid carcinoma:

1. Endometrioid carcinoma with squamous differentiation.
2. Endometrioid carcinoma with secretory features.
3. Endometrioid carcinoma with villoglandular pattern.
4. Endometrioid carcinoma with sertoliform pattern
5. Endometrioid carcinoma with microglandular pattern.

Approximately 10-25% of endometrioid adenocarcinoma show foci of squamous differentiation. The squamous differentiation are identified by intercellular bridges, keratin pearls, solid masses of polygonal cells with abundant eosinophilic glassy cytoplasm (61).

The endometrioid carcinomas are graded by the WHO based on the architecture (62).

Grade 1	Less than 5% non-squamous or non-morular growth pattern.
Grade 2	6%-50% non-squamous or non-morular growth pattern.
Grade 3	More than 50% non-squamous or non-morular growth pattern.

Table 5: Grading of endometrioid carcinoma

Tx	Primary tumour cannot be assessed.
T0	No evidence of primary tumour.
Tis	Carcinoma in situ
T1	Tumour confined to the corpus uteri
T1a	Tumour limited to endometrium or invading less than half of myometrium.
T1b	Tumour invades one half or more of myometrium.
T2	Tumour invades cervical stroma, but does not extend beyond the uterus
T3	Local and/regional spread
T3a	Tumour invades the serosa of the corpus uteri or adnexa(direct extension or metastasis)
T3b	Vaginal or parametrial involvement(direct extension or metastasis)
T4	Tumour invades bladder/bowel mucosa.
N1	Metastasis to pelvic or para-aortic lymph nodes.
N2	Metastasis to para-aortic lymph nodes with or without metastasis to pelvic lymph nodes
M1	Distant metastasis(exclude metastasis to vagina, pelvic serosa or adnexa)

Table 6: TNM staging of carcinomas of uterine endometrium

Genetic profile of endometrial carcinoma:

	Endometrioid	Serous	Carcinosarcoma
Bokhman subtype	I	II	II
TP53 mutation	Rare	>90%	60–90%
PI3K alterations	*PTEN* mutation (75–85%) *PIK3CA* mutation (50–60%) *PIK3R1* mutation (40–50%)	*PTEN* mutation (11%) *PIK3CA* amplification (45%) *PIK3CA* mutation (35%) *PIK3R1* mutation (12%)	*PTEN* mutation (19%) *PIK3CA* mutation (35%) *PIK3CA* amplification (14%)
KRAS mutation	20–30%	3%	17%
ERBB alterations	None	*ERBB2* amplification (25–30%)	*ERBB2* amplification (13–20%) *ERBB3* amplification or mutation (13%
FGFR amplification or mutation	*FGFR2* mutation (12%)	*FGFR2* mutation (5%) Frequent *FGFR1* and *FGFR3* amplification	*FGFR3* amplification (20%)
Wnt/β-catenin	*CTNNB1* mutation (25%)	*CTNNB1* mutation (3%)	..
Other	*ARID1A* mutation (35–40%)	*PPP2R1A* mutation (20%) *FBXW7* mutation (20% of undifferentiated endometrial carcinoma) *LRPB1* deletion Frequent amplifications in *MYC*, *CCNE1*, and *SOX17*	*PPP2R1A* mutation (28%) *FBXW7* mutation (35–40%) *ARID1A* mutation (25%) *CCNE1* amplification (42%) *SOX17* amplification (25%)

Table 7: Molecular classification of endometrial carcinoma (31)

Prognosis of endometrioid carcinoma:

The prognosis of endometrioid carcinoma is dependent mainly on the following factors: FIGO stage, age of the patient, stage of the disease, histological grade, depth of myometrial involvement and the lymphovascular invasion. The risk of recurrence is mainly dependent in the depth of myometrial invasion. Tumour involving more than half of the myometrium (outer half) is significantly associated with diminished survival rate in patients (63).

Survival rate of endometrial carcinoma:

The following data on survival rate of endometrial carcinoma patients is put forth by Creasman et al (n=5532 patients) (62).

Stage	Percentage
IA	91%
IB	88%
IC	81%
IIA	77%
IIB	67%
IIIA	60%
IIIB	41%
IIIC	32%
IVA	20%
IVB	5%

Table 8: Survival rate of patients with endometrioid carcinoma

Stage I[a]	Tumor contained to the corpus uteri		
	IA	No or less than half myometrial invasion	
	IB	Invasion equal to or more than half of the myometrium	
Stage II		Tumor invades the cervical stroma but does not extend beyond the uterus[b]	
Stage III[a]		Local and/or regional spread of tumor[c]	
	IIIA	Tumor invades the serosa of the corpus uteri and/or adnexas	
	IIIB	Vaginal and/or parametrial involvement	
	IIIC	Metastases to pelvis and/or para-aortic lymph nodes	
		IIIC1	Positive pelvic nodes
		IIIC2	Positive para-aortic lymph nodes with or without positive pelvic lymph nodes
Stage IV[a]			Tumor invades bladder and/or bowel mucosa and/or distant metastases
	IVA		Tumor invasion of bladder and/or bowel mucosa
	IVB		Disant metastases, including intra-abdominal metastases and or inguinal lymph nodes

Table 9: 2009 FIGO system for staging of carcinoma endometrium

Serous carcinoma:

Serous carcinoma is considered as the prototypical type II tumor. The prevalence of serous carcinoma is about 10% (64). The patients with serous carcinoma usually present a decade later than that of endometrioid adenocarcinoma. The patients are usually post-menopausal and more than 60 years. Patients with serous carcinoma are often multiparous, smokers and less obese compared to that of endometrioid carcinoma. History of tubal

ligation and past history of breast cancer are more often seen in cases of endometrial serous carcinomas (65).

Gross appearance:

These tumors are usually small and are associated with atrophic uterus. They may have an exophytic and papillary appearance. In few cases, a benign appearing polyp may be seen. Serous carcinoma and serous endometrial intraepithelial carcinoma are known to develop within a polyp (66).

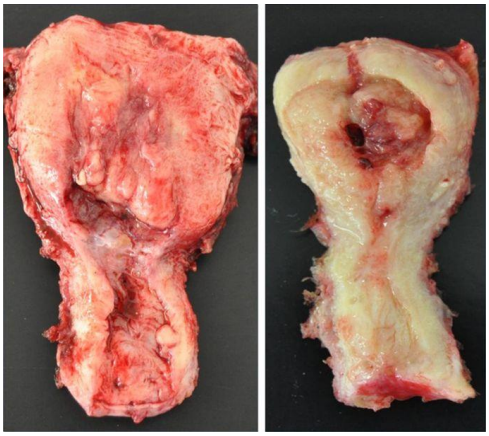

Figure 8: Gross appearance of serous carcinoma (67)

Histogenesis of serous carcinoma:

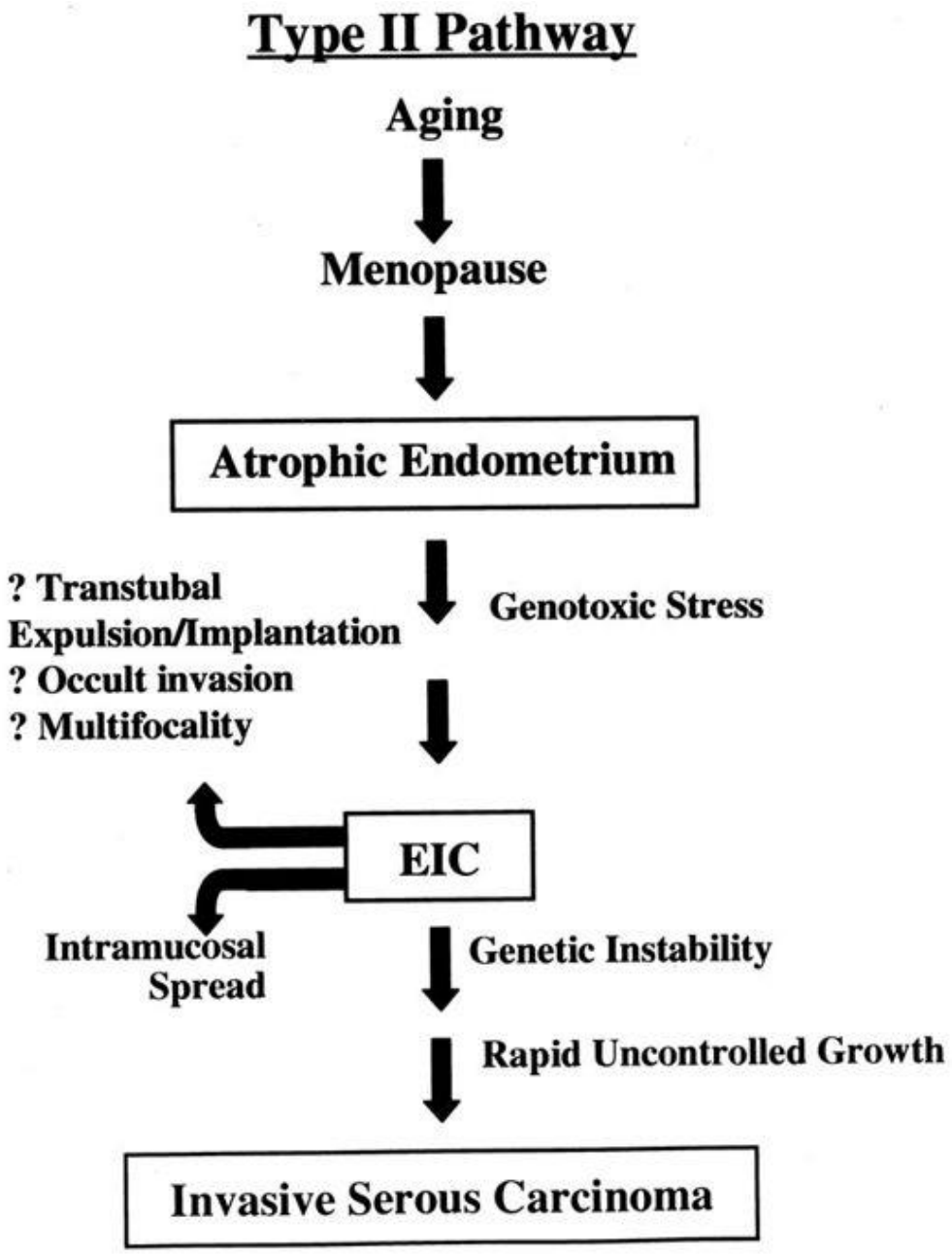

Figure 9: Histogenesis of Type II Pathway (41)

The precursor lesions for serous carcinoma is termed as ‘serous endometrial intraepithelial carcinoma (SEIC). They frequently develop on a polyp or in atrophic endometrium (68). On microscopy, SEIC often show a mild papillary architecture lined by columnar cells with enlarged, hyper chromatic smudged nuclei. Few cells can display hobnail morphology. There is brisk mitotic activity among the atypical cells. These lesions are diffusely positive for p53 on immunohistochemistry.

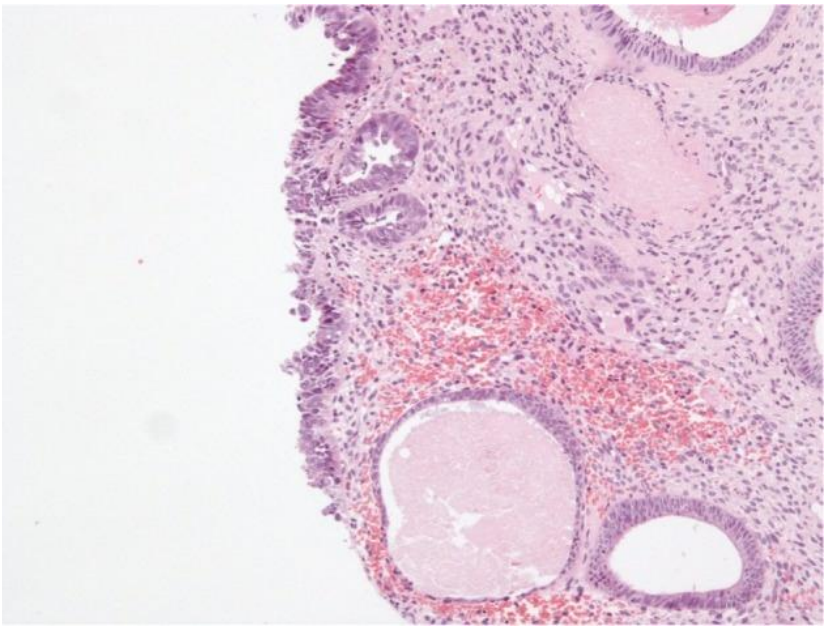

Figure 10: Serous endometrial intraepithelial carcinoma in a polyp (68)

The histological distinction between serous endometrial intraepithelial carcinoma and early invasive serous carcinoma is very difficult, and hence some authors prefer the term minimal uterine serous carcinoma (69).

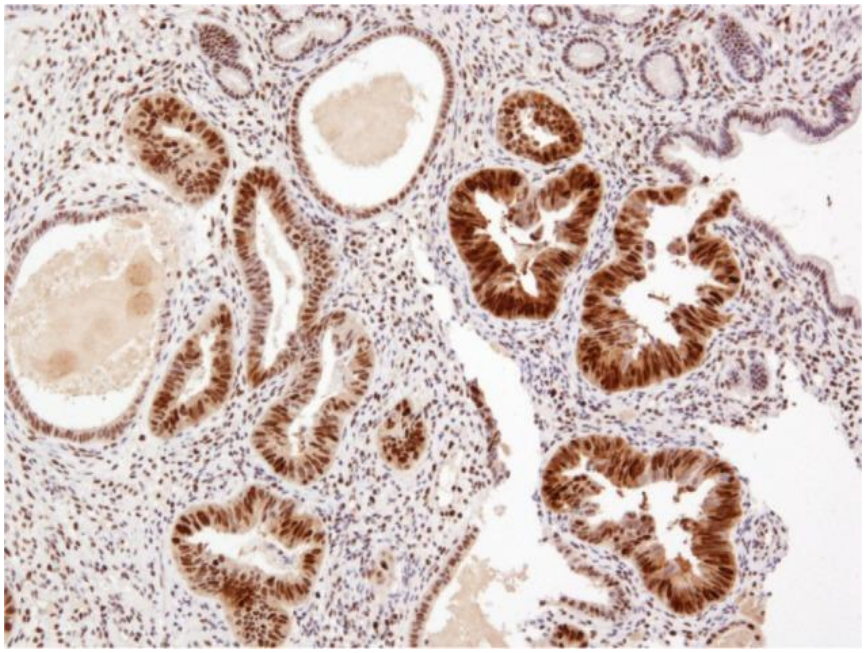

Figure 11: p53 staining in serous endometrial intraepithelial carcinoma (68)

Microscopy of uterine serous carcinoma:

The characteristic pattern associated with serous carcinoma is papillary architecture (short to long, branching) and lined by atypical polygonal cells with moderate to marked nuclear pleomorphism, hyperchromasia, macro nucleoli and moderate to abundant amounts of eosinophilic cytoplasm. Few of these tumours may have glandular or solid growth pattern (70).

Genetic profile of serous carcinoma:

The following are the most common somatic mutations in serous carcinoma of uterus:

Mutation	Percentage
TP53	80-90%
PIK3CA	24-40%
FBXW7	20-30%
PPP2R1A	18-28%

Table 10: The mutations associated with serous carcinoma (71)

Prognosis:

Serous carcinoma patients should be carefully staged because they may have extra-uterine spread even in the absence or very minimal myometrial invasion. The involvement of extra uterine sites such as ovary, peritoneum and fallopian tubes can occur very early in the course of disease compared to other endometrial adenocarcinomas.

According to Abeler et al, the 5 year and 10 year survival rates(all stages of tumour) of serous carcinoma are 36% and 18% respectively (64). The prognostic factors for shorter survival are as follows:

1. Age more than 60 years.
2. Vascular invasion.
3. Myometrial invasion- more than 50%.

Carcinosarcoma:

Carcinosarcoma [Malignant Mullerian Mixed Tumors (MMMT)] comprise less than 5% of all the malignancies in the uterus (72). By definition, carcinosarcoma is a biphasic tumour composed of high grade carcinomatous and sarcomatous elements (48). The mean age of patients presenting with carcinosarcoma is about 70 years. The risk factors for carcinosarcoma are difficult to determine because of the low prevalence of the disease. Most of the studies proposed that patients with carcinosarcoma and endometrioid adenocarcinoma share the same risk factors.

Gross:

Carcinosarcomas are mostly polypoidal and fills the entire uterine cavity. In about half of the patients, the tumour protrudes through the cervix (73). The tumour has a fleshy, soft to firm tan cut surface.

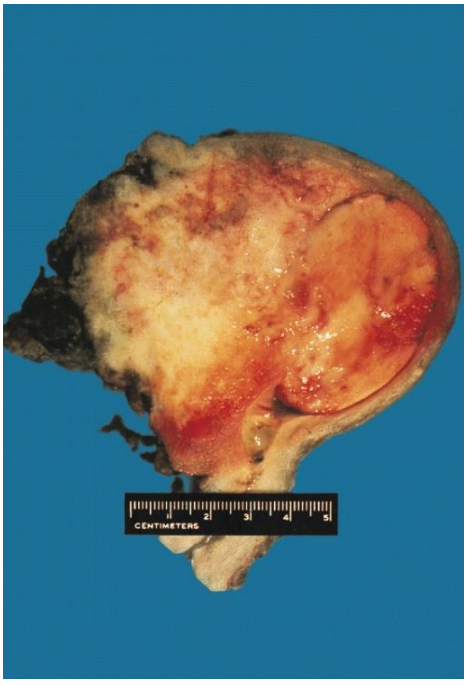

Figure 12: Gross picture of carcinosarcoma (73)

Histogenesis:

MMMT likely represent carcinomas with a mesenchymal component as a result of metaplasia and tumour progression. The recent clinico-pathological, immunohistochemical and molecular findings suggest that these tumors arise from a single multipotent stem cell, by a process called 'bidirectional differentiation' (74).

Microscopy:

Carcinosarcomas are composed of a mixture of high grade epithelial and mesenchymal components. The epithelial component is composed mostly of endometrioid carcinoma, but other subtypes such as serous, mucinous, squamous and mesonephric carcinomas can also be seen (75). The mesenchymal component is composed of a high grade sarcoma. Heterologous differentiation such as chondrosarcoma, osteosarcoma and rhabdomyosarcoma is seen in around 50% of the cases (76).

Genetic profile:

Many genetic and molecular studies have confirmed that similar molecular pathways are involved in the pathogenesis of endometrioid carcinoma and carcinosarcoma. The phenotypic characteristic of carcinosarcoma is dependent on changes in Akt/β catenin pathway and repression of E- Cadherin (77).

Prognosis:

Carcinosarcomas are associated with poor outcome with a five year survival of 15%-30% in advanced stage disease (78). The prognosis is dependent on the following factors:

1. Advanced age.

2. Extra uterine extension
3. Deep myometrial invasion.
4. Presence of serous or clear cell carcinoma as epithelial component.
5. Presence of heterologous sarcomatoid elements.

Treatment of endometrial carcinomas:

The main stay of treatment for endometrial carcinoma is surgery, followed by adjuvant therapy such as radiotherapy and chemotherapy. Few targeted therapies are also available.

The standard treatment for endometrioid carcinoma is surgery (Total hysterectomy and bilateral salpingo-oophorectomy). Post-operative progestin therapy is given for patients with no significant poor prognostic factors (79). Pelvic and para-aortic sampling should be done in patients with the following features (80):

1. More than 50% myometrial invasion
2. Grade 3 tumour.
3. Involvement of cervix.
4. Presence of extra uterine spread.
5. Palpably enlarged lymph nodes.

When treated with postoperative radiotherapy, the five year survival rate of women with positive lymph nodes is improved to 40%.

The current approach in the treatment of uterine serous carcinoma is hysterectomy with bilateral salpingo-oophorectomy, omentectomy, peritoneal cytology, pelvic

and para-aortic lymph node sampling. Because of the aggressive behavior of serous carcinoma, adjuvant therapy should always be considered. Few studies show that platinum based chemotherapy is useful in patients with high grade serous carcinoma (81).

Carcinosarcomas are treated by total hysterectomy and bilateral salpingo-oophorectomy with lymph node dissection, peritoneal and pelvic washings. The adjuvant therapies that was recently approved by the Gynecologic Oncology Group is the use of combination chemotherapy (cisplatin, ifosfamide and mesna) over abdominal radiotherapy (82).

Recent advances in endometrial cancer:

The understanding of molecular pathway in the pathogenesis of endometrial carcinoma help us to guide in the development of targeted therapies for its treatment. The following are the new targeted therapies available for the treatment of endometrial carcinoma (85):

1. Anti-angiogenic agents.
2. EGFR-Inhibitors.
3. HER2/neu antibodies
4. mTOR pathway inhibitors.

MATERIALS AND METHODS:

This study was done in The Department of General Pathology. Cases diagnosed as disordered proliferation, typical and a typical hyperplasia on biopsy and resection specimens of endometrial adenocarcinoma, serous and carcinosarcoma during the time period of January 2014- December2016 are included in this study.

Inclusion criteria:

- Cases diagnosed as disordered proliferative endometrium (DPEM), typical and atypical hyperplasia in curettings and subsequently underwent hysterectomy.
- Cases which underwent hysterectomy for endometrial adenocarcinoma, serous carcinoma and carcinosarcoma.

Exclusion criteria:

- Cases of disordered proliferative endometrium (DPEM), typical and atypical hyperplasia in curettings with no subsequent hysterectomy.
- Blocks handed over to the patients.
- Biopsy sample inadequate to proceed with immunohistochemistry.
- Referred cases with only slides submitted for review.

Methodology:

The clinical details of all the patients such as age, post-menopausal status, duration of post-menopausal status, clinical presentation, endometrial thickness, parity, history of exogenous hormone intake, family history of tumours, syndromic association, Body Mass Index (BMI) and comorbidities were retrieved from the Medical Records Department.

Stage, grade, presence/ absence of lymphovascular invasion and lymph node metastasis if any were retrieved from the pathology work station.

Assessment of ER and PR on all the cases was done by immunohistochemistry.

Precursor lesions:

The total number of cases diagnosed as disordered proliferation were 503. Out of which 30 cases had both curettings and resection specimens. Nine blocks were unavailable and hence 21 cases were included in this study. The total number of cases diagnosed as typical hyperplasia were 125. Out of which 32 cases had both resection and curettings. Three blocks were unavailable and hence 29 cases were included in this study. The total number of cases with atypical hyperplasia were 39. Fourteen blocks were unavailable and hence 25 cases were included in this study.

Endometrial carcinomas:

The total number of endometrioid carcinomas in resection specimen were one forty two patients (n=142). The cases were randomized and twenty cases were selected for this study. There were eight cases of primary uterine serous carcinomas (n=8). Out of which blocks were unavailable for 4 cases. There were eighteen cases of carcinosarcoma (n=18). Out of which blocks were unavailable for four cases.

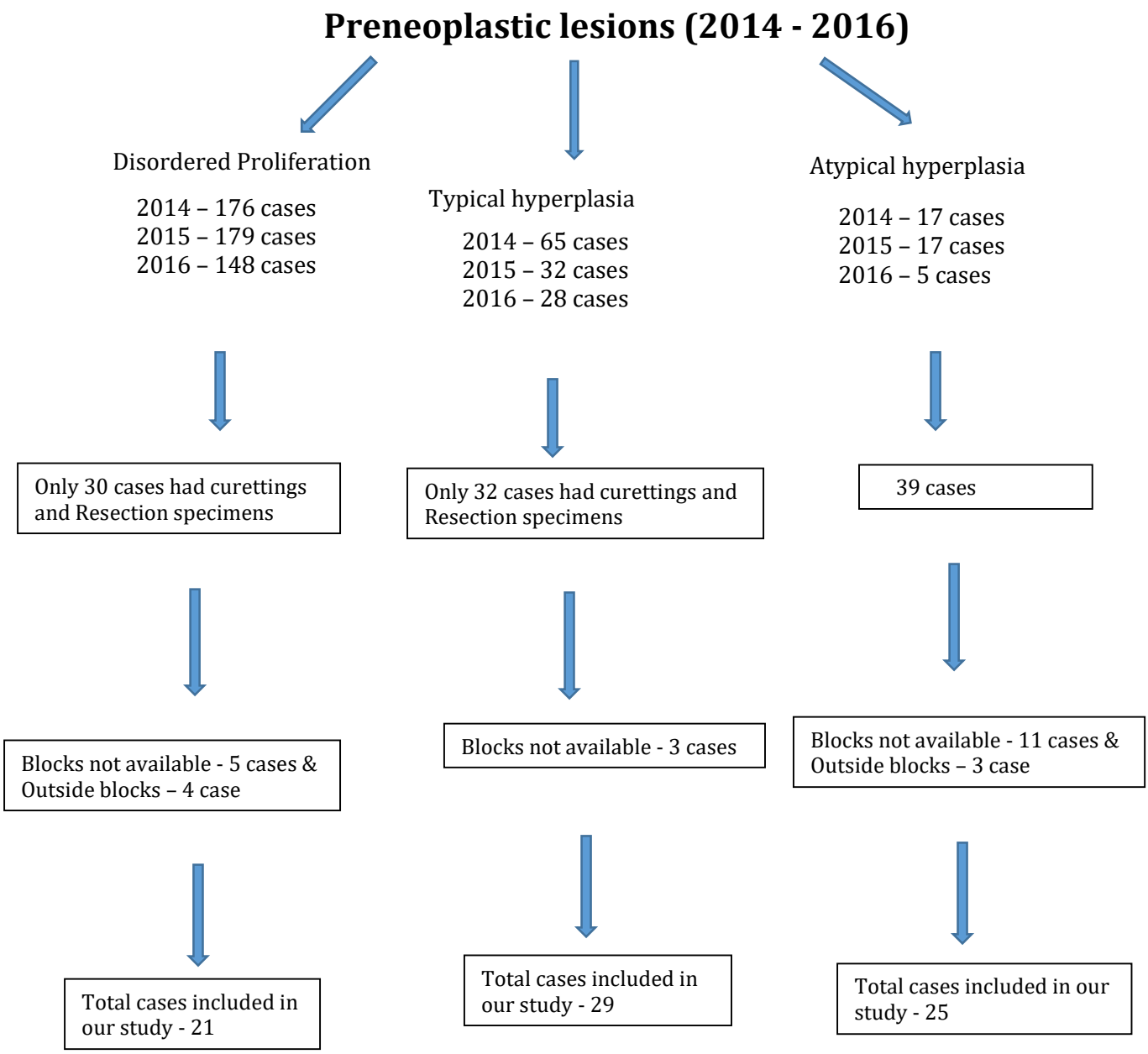

Figure 13: Schema figure for preneoplastic lesions

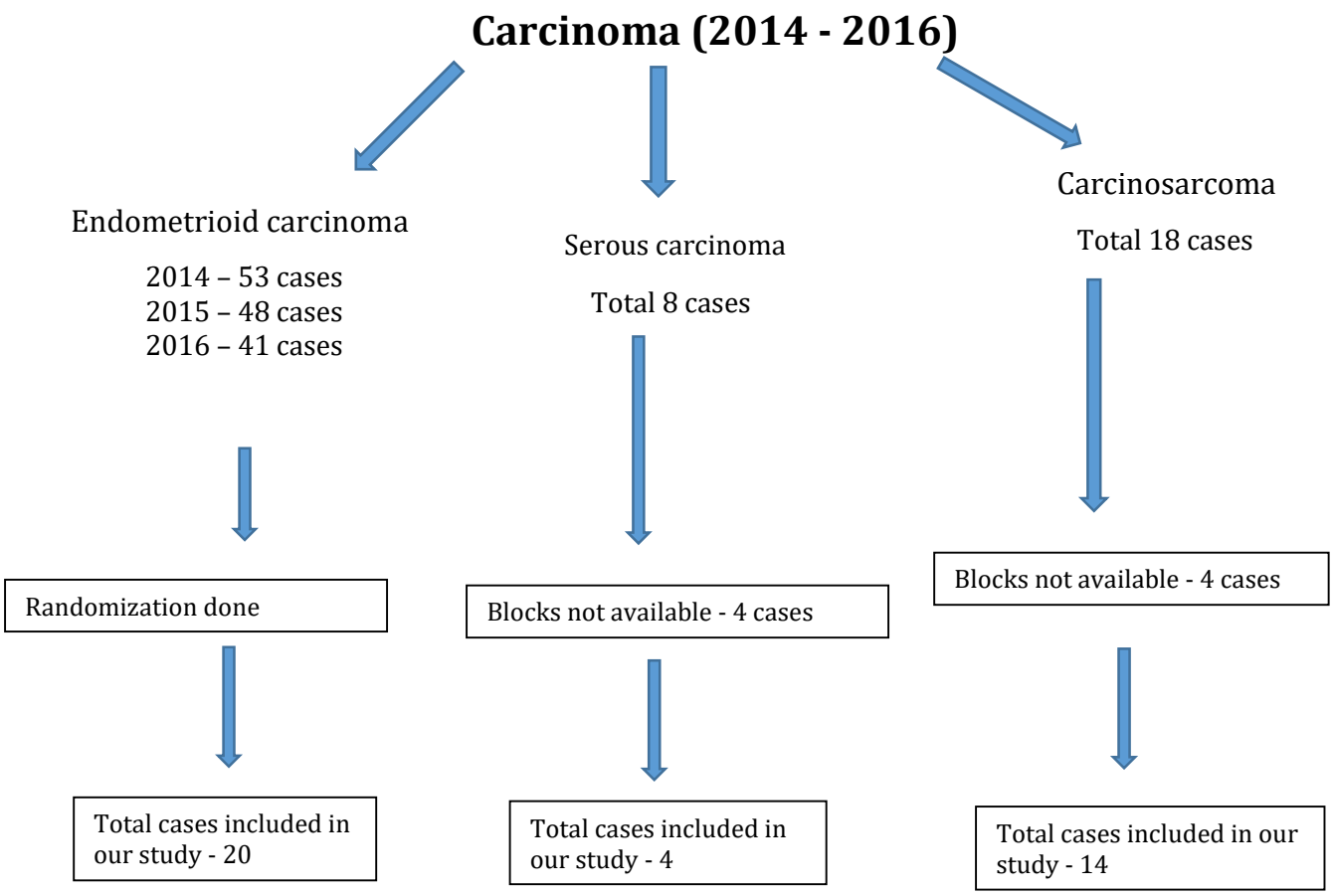

Figure 14: Schema figure for endometrial carcinoma

Study group	Number of cases
Disordered proliferation (DPEM)	21
Typical hyperplasia	29
Atypial hyperplasia	25
Endometrioid carcinoma	20
Serous carcinoma	04
Carcinosarcoma	14

Table 11: Total number of cases in this study

Immunohistochemical evaluation of ER and PR was performed according to the method described by Carcangiu ML et al., based on the percentage of stained cells and the intensity of nuclear stain.

Score	Percentage of nuclei stained
1	0% to 25% of the nuclei
2	26% to 75% of nuclei
3	more than 76% of the nuclei

Table 12: Grading the percentage of stained cells

Score	Intensity
1	absent or weak
2	Strong
3	very strong

Table 13: Grading the staining intensity of the cells

The sum of both parameters gave the immunohistochemical score.

Tumours were divided into three categories depending on the immunohistochemical score. Category I tumours were considered as immune-negative, whereas Category II and III tumours were considered as immune-positive.

Category I corresponded to a score of 2,

Category II corresponded to a score of 3 or 4,

Category III corresponded to a score of 5 or 6.

The procedure for immunohistochemistry and the clone of antibody used in assessing the ER and PR expression is given in Appendix 1.

RESULTS:

Clinical features:

Disordered proliferation:

In disordered proliferation, two out of twenty one patients (9%) were postmenopausal. The age distribution of disordered proliferation was 39-53 years. The most common clinical presentation in this group (85.71%) was perimenopausal bleeding. According to WHO, fourteen out of twenty cases (70%) had above normal body mass index (BMI). Nine out of twenty one cases (42%) were associated with a history of exogenous hormone intake. The average endometrial thickness in this group ranged from 0.5cm - 5.1cm. Three out of twenty one patients (14%) were associated with family history of other tumours.

Typical hyperplasia:

In typical hyperplasia, twenty out of twenty nine patients (68%) were postmenopausal. The age distribution of typical hyperplasia was 37-70 years. The most common clinical presentation in this group (66%) was postmenopausal bleeding. According to WHO, twenty five out of twenty-nine cases (86%) had above normal BMI. Ten out of twenty nine cases (34%) were associated with a history of exogenous hormone intake. The average endometrial thickness in this group ranged from 0.4cm – 2.5cm. Three out of twenty nine patients (10%) were associated with family history of other tumours.

Atypical hyperplasia:

In atypical hyperplasia, thirteen out of twenty five patients (52%) were post-menopausal. The age distribution of atypical hyperplasia was 29-74 years. The most common clinical presentation in this group (52%) was postmenopausal bleeding. Seventeen out of twenty cases (85%) had above normal BMI. The BMI of five patients was not available. Ten out of twenty three cases (39%) were associated with a history of exogenous hormone intake. The average endometrial thickness in this group ranged from 0.5cm – 5.3cm. Two out of twenty five patients (12%) were associated with family history of other tumours.

Endometrial carcinoma:

In endometrial carcinoma, fifteen out of twenty patients (75%) were postmenopausal. The age distribution of endometrial carcinoma was 34-71 years. The most common clinical presentation in this group (75%) was postmenopausal bleeding. Ten out of nineteen cases (52%) had above normal BMI. The BMI of one patient was not available. Two out of twenty cases (10%) were associated with a history of exogenous hormone intake. The average endometrial thickness in this group ranged from 0.8cm – 7.9cm. Five out of twenty patients (25%) were associated with family history of other tumours.

Serous carcinoma:

In serous carcinoma, all the four patients were post-menopausal. The age distribution of serous carcinoma was 50-60 years. All the patients in this group presented with postmenopausal bleeding. None of the patients had a history of exogenous hormone intake.

Carcinosarcoma:

In carcinosarcoma, thirteen out of fourteen patients were (92%) were postmenopausal. The age distribution of carcinosarcoma was 49-77 years. The most common clinical presentation in this group (93%) was postmenopausal bleeding. Ten out of fourteen cases (71%) had above normal BMI. The average endometrial thickness in this group ranged from 1.0cm – 7.8cm. Two out of fourteen cases (14%) were associated with family history of other tumours.

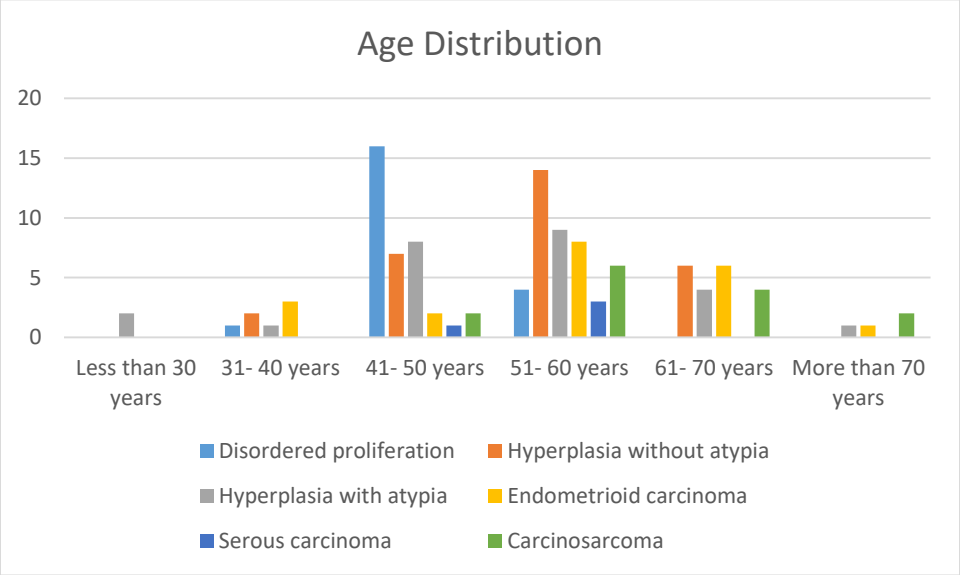

Figure 15: Age distribution of endometrial preneoplastic lesions and carcinomas

The mean age of presentation of preneoplastic lesions of endometrium was 51 years. In preneoplastic lesions, the frequency of disordered proliferation was a decade earlier than other lesions. In this study, atypical hyperplasia was randomly distributed among all age

groups. The most common age group for carcinomas was above 50 years. The mean age of carcinomas was 58 years. Endometrioid carcinoma was seen in a decade earlier than serous and carcinosarcoma. In summary, the preneoplastic lesions occurs at a relatively younger patients compared to carcinomas.

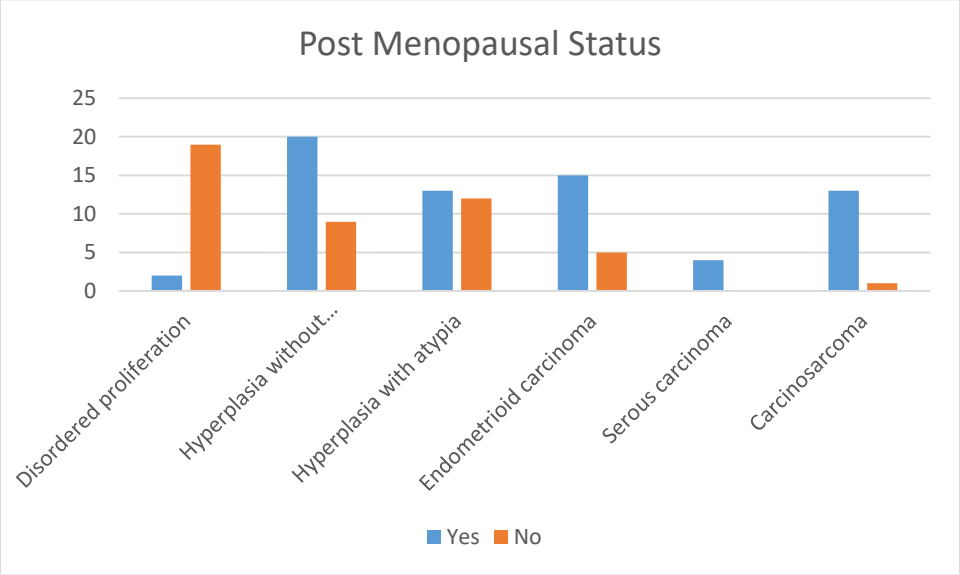

Figure 16: Postmenopausal status of endometrial preneoplastic lesions and carcinomas

This table suggests disordered proliferation was more frequently associated with pre and perimenopausal age group. On the other hand, carcinomas were more frequently associated with post-menopausal status.

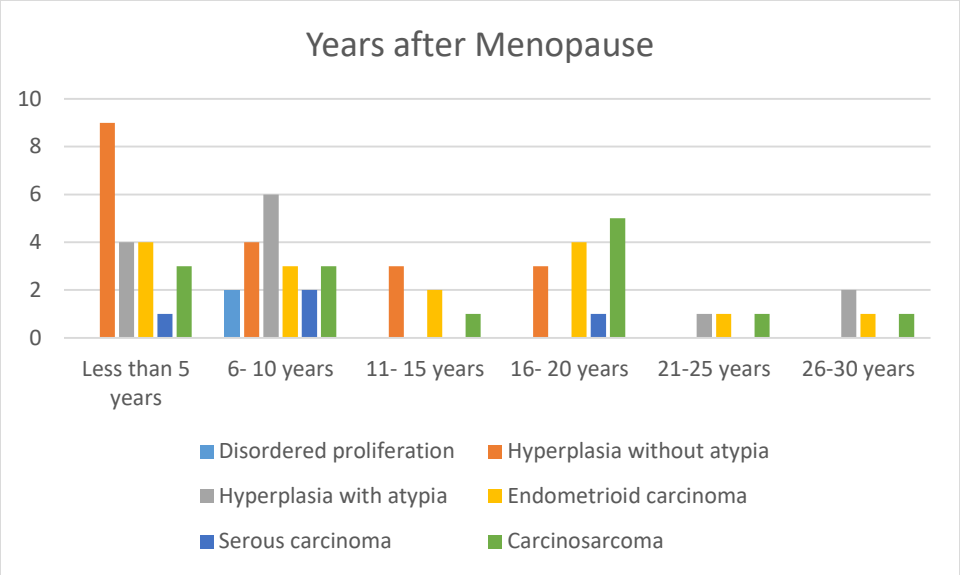

Figure 17: Number of years after menopause

Presenting complaints:

The presenting complaints included post-menopausal bleeding, abnormal uterine bleeding, abdominal pain, and others including white discharge per vagina, primary infertility, itching and urinary retention. Three patients of atypical hyperplasia presented with primary infertility. Overall the most common clinical presentation was post-menopausal bleeding.

Clinical presentation	Number of patients (n=113)
Post-menopausal bleeding	55
Abnormal uterine bleeding	46
Abdominal pain	7
Others	14

Table 14: Clinical presentation of preneoplastic lesions and endometrial carcinomas

Parity:

Five out of thirty three patients with carcinoma were nulliparous. Seven out of seventy one patients with preneoplastic lesions were nulliparous. The parity status of nine patients was not known. Altogether, the incidence of carcinoma is 34.02 % and 41.67% in parous and nulliparous women respectively. Three out of fourteen carcinosarcoma patients were nulligravida (21%).

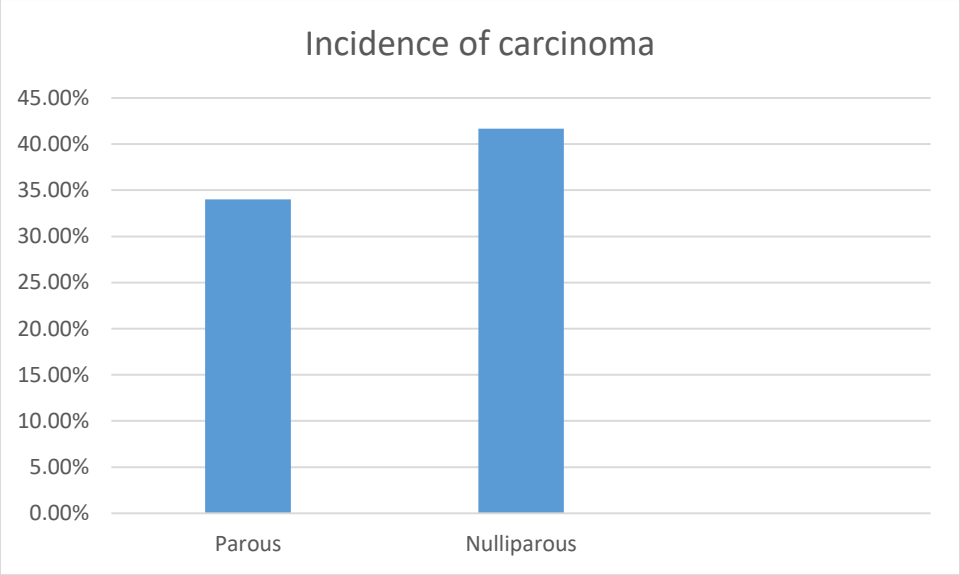

Figure 18: The incidence of carcinoma in nulliparous women

Body mass index:

The body mass index (BMI) of the patients was classified according to the WHO.

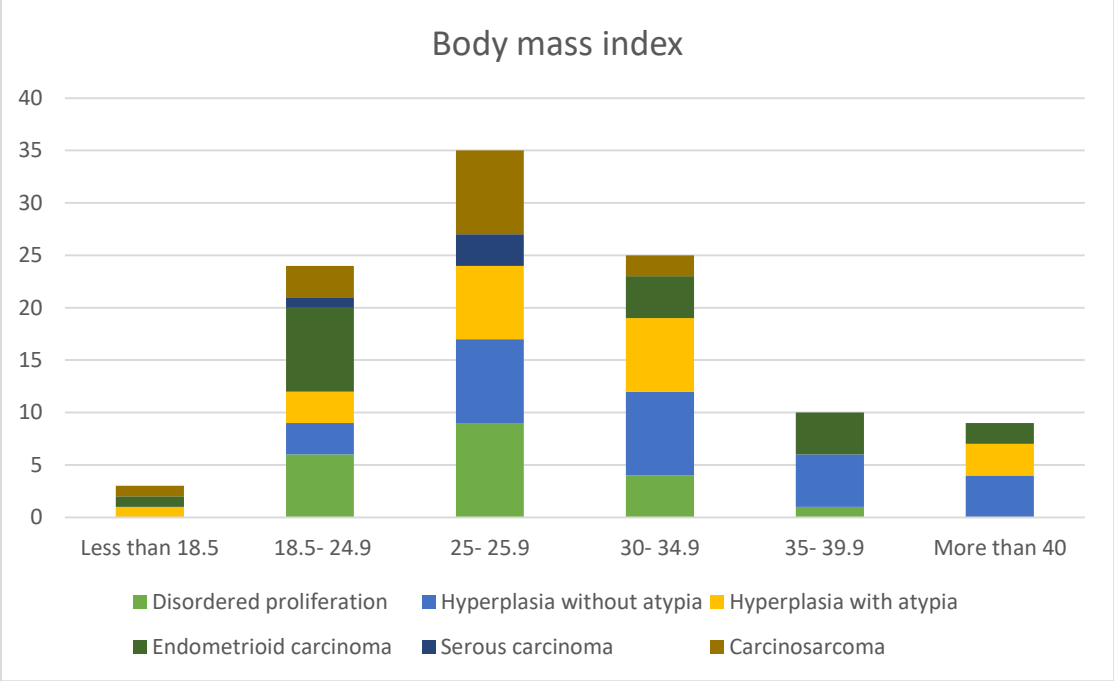

Figure 19: Body mass index of preneoplastic lesions and carcinoma

In preneoplastic lesions, 1% were underweight, 17% were normal weight, 35% were overweight and 46% were obese. BMI of six patients was not available.

In carcinoma, 5% were underweight, 32% were normal weight, 30% were overweight and 32% were obese. BMI of one patient was not available.

Comorbidities:

The comorbidities associated with the study population were diabetes mellitus, hypertension, hypothyroidism and others. Others conditions included coronary heart disease, seizure, obstructive sleep apnoea and respiratory disorders.

Comorbidities	Number of patients (n=113)
Diabetes	41
Hypertension	43
Hypothyroidism	12
Others	22
Nil	42

Table 15: Comorbidities associated with preneoplastic lesions and carcinomas

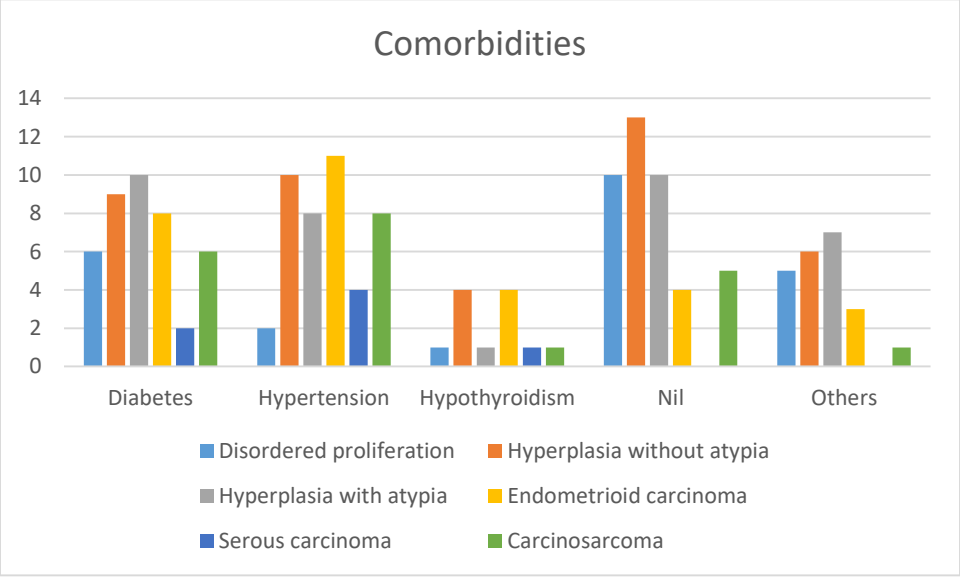

Figure 20: Comorbidities associated with preneoplastic lesions and carcinomas

Diabetes and hypertension were the most common comorbidities associated with the study population. Most of the patients with preneoplastic lesions were without any comorbidities, probably as they presented at a younger age.

Association with exogenous hormones:

The incidence of carcinoma in patients who took hormonal therapy was 9.68% in our study population. The history of hormonal use was seen mostly among patients with preneoplastic lesions. Twenty eight out of seventy two patients (39%) with preneoplastic lesions had history of exogenous hormone intake. History of hormonal intake in three patients was not available.

History of other tumours:

In preneoplastic lesions, nine out of seventy five patients (12%) had family history of other tumours. In cases of carcinoma, seven out of thirty eight patients (19%) had family history of other tumours.

Endometrial thickness:

The mean endometrial thickness in preneoplastic lesions was 13.27mm. The mean endometrial thickness in carcinomas was 26.13mm. The endometrial thickness was less than or equal to five in only seven patients (7%). Among the seven, five cases belonged to preneoplastic group and remaining two belonged to tumour group.

Parameter	Endometrial preneoplastic lesions (n=75)	Endometrial carcinoma (n=38)	P value*
Mean age	50.92+/- 9.05	58.11+/- 8.99	<0.001
Postmenopausal Yes No	 35(46.67%) 40(53.33%)	 32(84.21%) 6(15.79%)	<0.001
Parity Gravida Nulligravida	 64(90.14%) 7(9.86%)	 33(86.84%) 5(13.16%)	0.600
History of tumour Yes No	 9(12.16%) 65(87.54%)	 7(18.42%) 31(81.58%)	0.370
History of exogenous hormones Yes No	 28(38.89%) 44(61.11%)	 3(7.89%) 35(92.11%)	0.001
Presenting complaints PMB AUB Abdominal pain Others	 31(41.33%) 39(52%) 3(4%) 5(6.67%)	 24(63.16%) 7(18.42%) 4(10.53%) 9(23.68%)	 0.028 0.001 0.174 0.009
Comorbidities Hypothyroid	 6(8%)	 6(15.79%)	0.204

Diabetes mellitus	25(33.33%)	16(42.11%)	0.360
Hypertension	21(28%)	23(60.53%)	0.001
Nil	35(46.67%)	9(23.68%)	0.018
Others	15(20%)	4(10.53%)	0.203
Endometrial thickness	13.27+/-9.65	26.13+/- 21.20	<0.001

*p value of less than 0.05 is considered as statistically significant.

Table 16: Comparison of clinical and radiological features between endometrial preneoplastic lesions and carcinoma

Tumour size:

The mean tumour size was 4.84cm. The largest tumor in this study population was 11.5cm (endometrioid carcinoma, grade 3, Stage I). All the patients with carcinosarcomas had a tumour size of more than or equal to three.

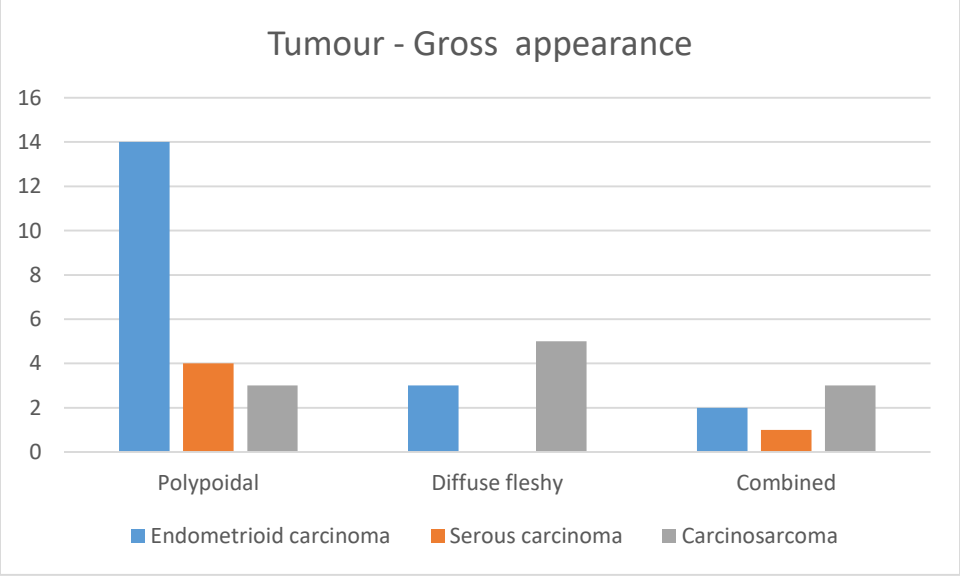

Figure 21: Gross appearance of tumours

Endometrioid carcinomas had polypoidal/proliferative friable growth in the endometrial cavity. Carcinosarcomas and serous carcinoma were bulky polypoidal masses.

Grading of carcinoma:

The endometrioid carcinomas were graded according to the WHO system of classification.

Grade	No. of cases (n=20)
Well differentiated	6
Moderately differentiated	8
Poorly differentiated	6

Table 17: Grading of endometrioid carcinoma based on FIGO system

Depth of invasion:

The mean depth of invasion in tumour was 8.16mm. In twenty two out of thirty eight patients (58%) less than half of the myometrium was involved. In seven out of fourteen patients (50%) of carcinosarcoma, more than half of the endometrium was involved.

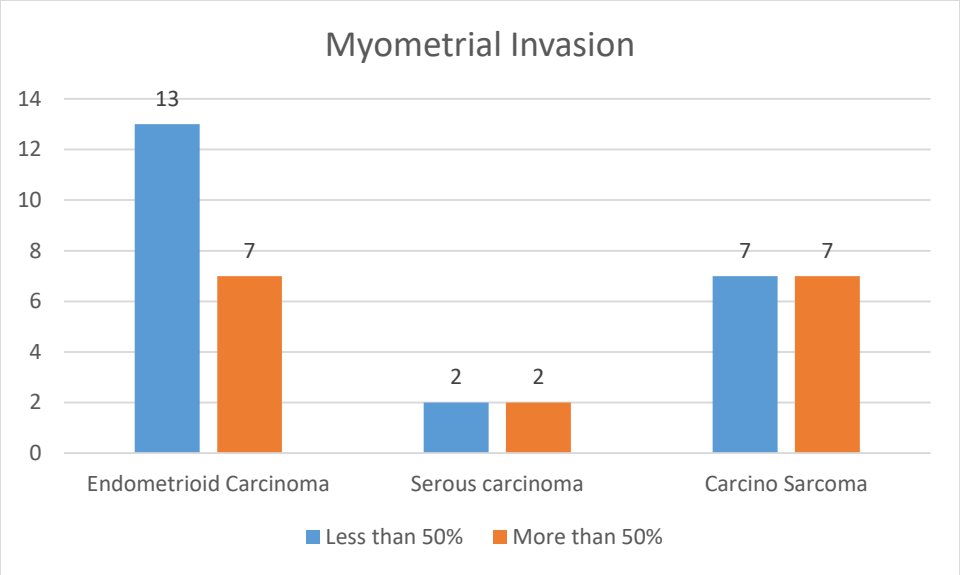

Figure 22: Depth of myometrial invasion in endometrial carcinomas

Lymphovascular invasion:

Lymphovascular invasion was present in nine out of thirty eight cases of carcinomas (24%). In endometrioid carcinoma, three out of twenty patients (15%) had lymphovascular invasion. In carcinosarcoma, six out of fourteen patients (43%) had lymphovascular invasion. The most common lymph node group involved were left and right pelvic lymph nodes. None of the cases of serous carcinoma had lymphovascular invasion.

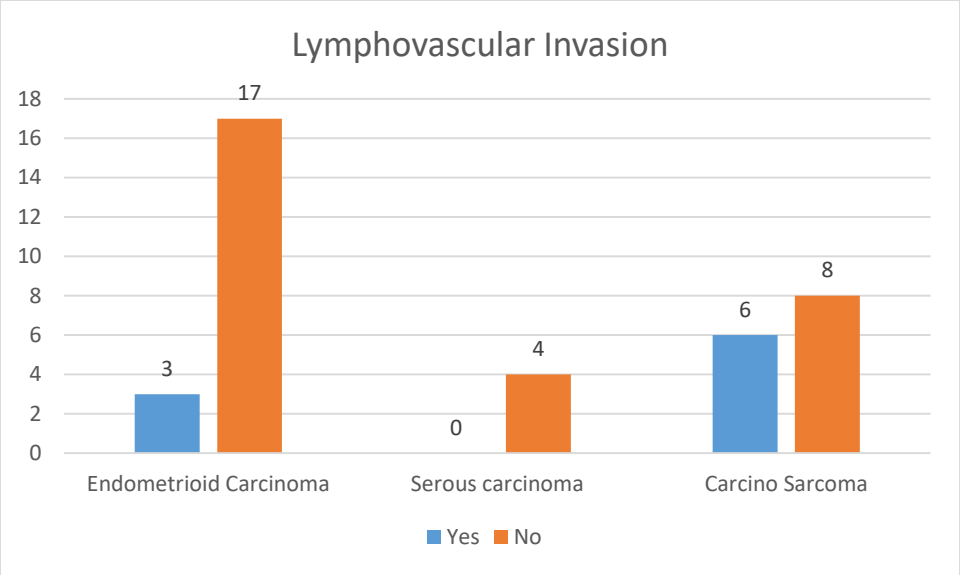

Figure 23: Lymphovascular invasion in endometrial carcinoma

Stage of the tumour:

The tumour stage is based on TNM classification.

Stage	Number of cases
1	30
III	7
IV	1

Table 18: Distribution of cases according to the TNM staging

None of the cases in this study belonged to stage II. In endometrioid carcinoma, seventeen out of twenty cases (85%) had stage I disease. In carcinosarcoma eleven out of fourteen cases (79%) had stage I disease. In serous carcinoma two cases each had stage I& stage III disease.

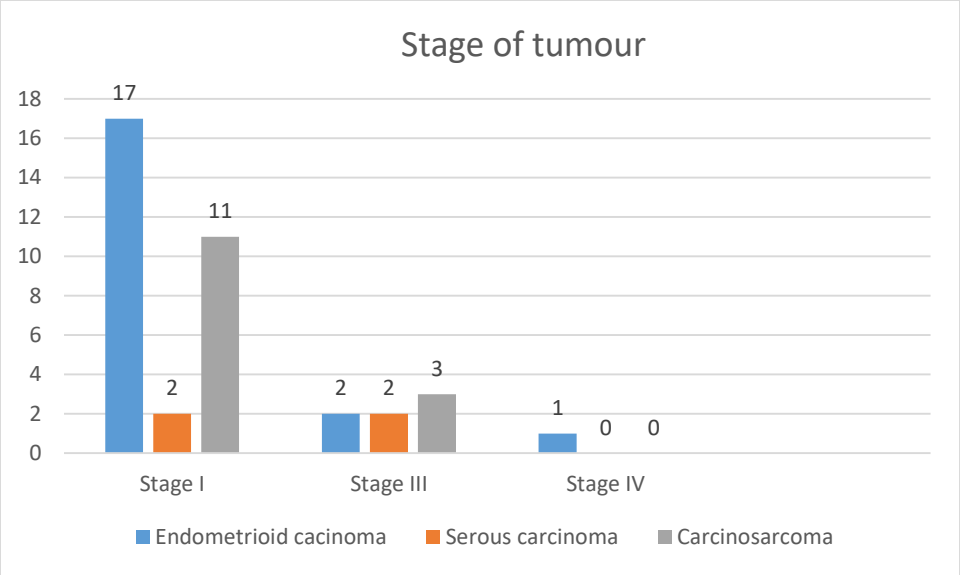

Figure 24: Stage of tumour

Other associated lesions:

Fourteen out of thirty eight cases (37%) showed other associated benign lesions such as leiomyoma, adenomyosis and endometriosis. Two out of twenty (10%) of the endometrioid carcinoma were associated with endometriosis.

Associated lesions	No. of cases (n=14)
Leiomyoma	8
Adenomyosis	4
Endometriosis	2

Table 19: Lesions associated with endometrial carcinomas in resection specimen

Association with heterologous elements in carcinosarcoma:

Six out of fourteen cases of carcinosarcoma (43%) were associated with heterologous elements. Out of six cases, four cases had cartilaginous differentiation and two cases had skeletal muscle differentiation.

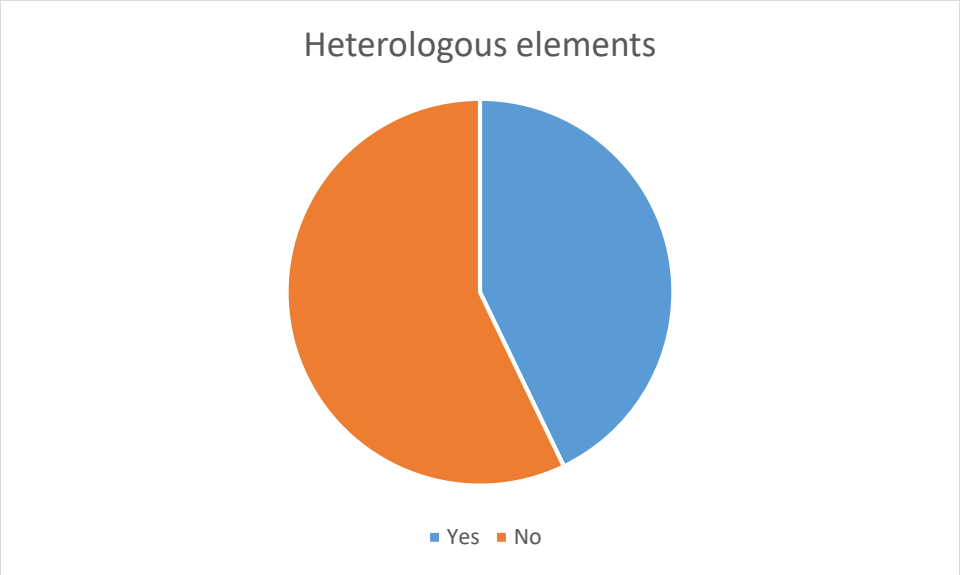

Figure 25: Heterologous elements in carcinosarcoma

Immunohistochemical expression of ER and PR:

ER:

Out of the 38 cases of carcinoma, 14 cases (37%) belonged to category I, 16 cases (42%) belonged to category II and 8 cases (21%) belonged to category III. All cases of endometrioid carcinomas belonged to category III.

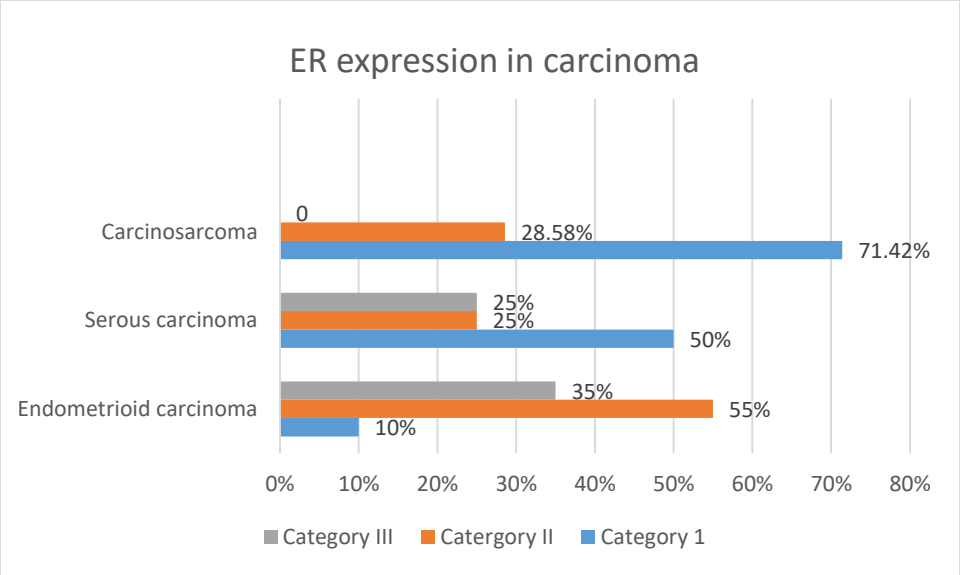

Figure 26: ER expression in endometrial carcinoma

PR:

Among the 38 cases of tumour, 20 cases (53%) belonged to category I, 8 cases (21%) belonged to category II and 10 cases (26%) cases belonged to category III. All the cases of carcinosarcoma belonged to category I.

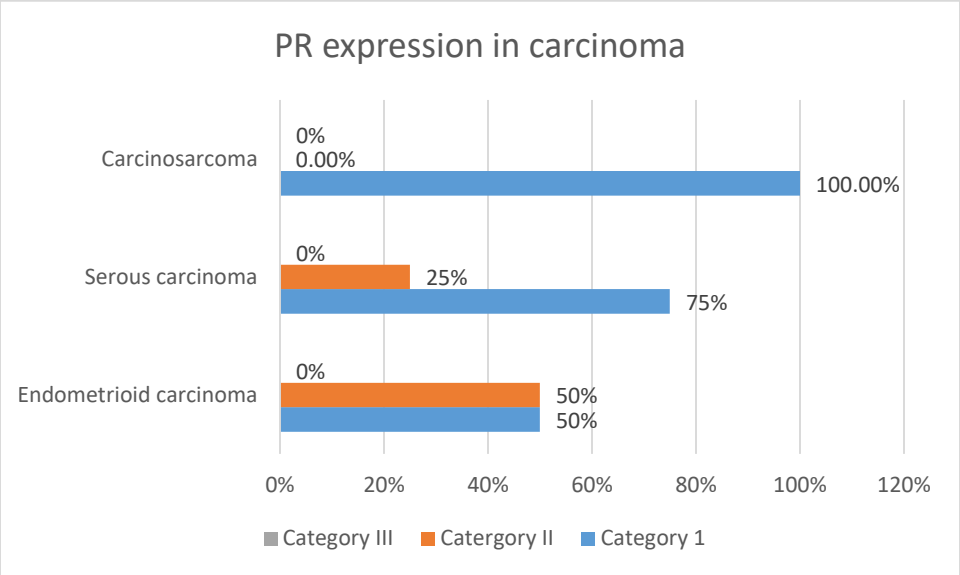

Figure 27: PR expression in endometrial hyperplasia

Expression of ER and PR in precursor lesions:

ER:

Out of the 75 cases, only one case (1%) belonged to category I, 6 cases (8%) belonged to category II and 68 cases (91%) belonged to category III. Out of the six cases in category II, five cases were of atypical hyperplasia. Out of the 5 cases, one case was diagnosed as complex hyperplasia with atypia in endometrial curettings and endometrioid carcinoma (grade 1) in resection specimen.

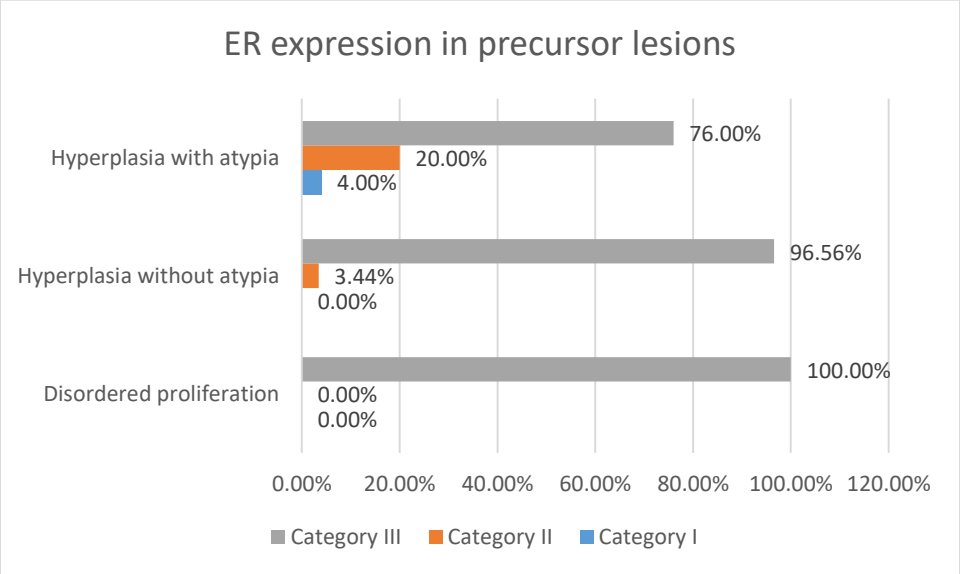

Figure 28: ER expression in preneoplastic lesions

PR:

Among the 75 cases, only one case (1%) belonged to category I, one case (1%) belonged to category II and remaining 73 cases belonged to category III (97%).

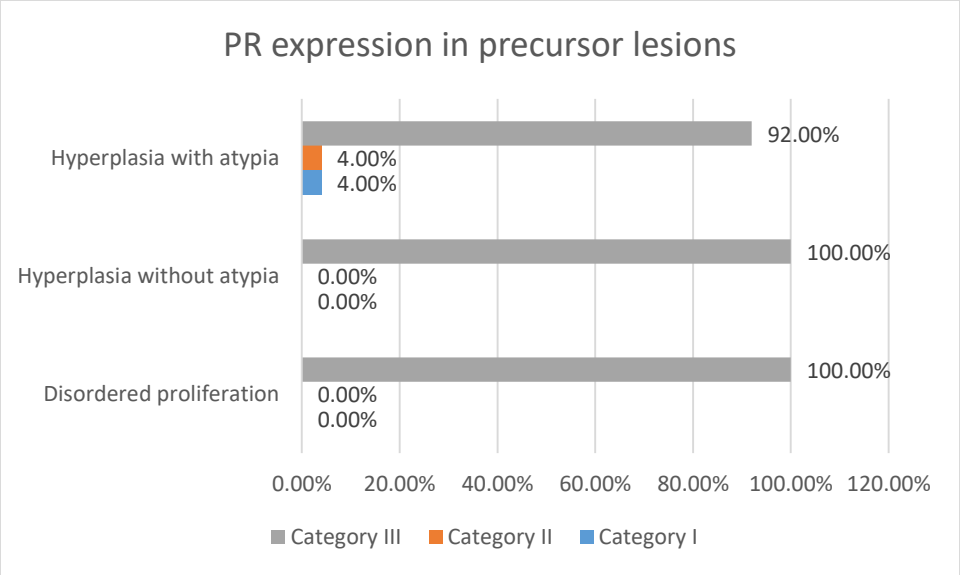

Figure 29: PR expression in preneoplastic lesions

Parameter	ER Category			ER p value*
	1	2	3	
Stage				
1	9(64.29%)	13(81.25%)	8(100%)	0.173
3 – 4	5(35.71%)	3(18.75%)	0	
Grade				
Well differentiated	0	1(6.25%)	5(62.5%)	< 0.001
Moderately differentiated	1(7.14%)	7(43.75%)	2(25%)	
Poorly differentiated	13(92.86%)	8(50%)	1(12.5%)	
Lymphovascular Invasion				
Yes	6(42.86%)	3(18.75%)	0	0.06
No	8(57.14%)	13(81.25%)	8(100%)	
Diagnosis				
Disordered Proliferation	0	0	21(27.63%)	< 0.001
Typical hyperplasia	0	1(4.55%)	28(36.84%)	
Atypical hyperplasia	1(6.67%)	5(22.73%)	19(25%)	
Endometrioid Carcinoma	2(13.33%)	11(50%)	7(9.21%)	
Serous Carcinoma	2(13.33%)	1(4.55%)	1(1.32%)	
Carcinosarcoma	10(66.67%)	4(18.18%)	0	
Endometrial Thickness				
Less than 5 mm	0	1(5.26%)	6(8.96%)	0.841
More than 5 mm	12(100%)	18(94.74%)	61(91.04%)	

Table 20: Comparison of ER expression with histological parameters

Parameter	PR Category			PR p value
	1	2	3	
Stage				
1	13(65%)	7(87.5%)	10(100%)	0.066
3 – 4	7(35%)	1(12.5%)	0	
Grade				
Well differentiated	0	2(25%)	4(40%)	< 0.001
Moderately differentiated	2(10%)	3(37.5%)	5(50%)	
Poorly differentiated	18(90%)	3(37.5%)	1(10%)	
Lymphovascular Invasion				
Yes	8(40%)	1(12.5%)	0	0.025
No	12(60%)	7(87.5%)	10(100%)	
Diagnosis				
Disordered Proliferation	0	0	21(25.30%)	< 0.001
Typical hyperplasia	0	0	29(34.94%)	
Atypial hyperplasia	1(4.76%)	1(11.11%)	23(27.71%)	
Endometrioid Carcinoma	5(23.81%)	6(66.67%)	9(10.84%)	
Serous Carcinoma	3(14.29%)	0	1(1.2%)	
Carcinosarcoma	12(57.14%)	2(22.2%)	0	
Endometrial Thickness				
Less than 5 mm	0	1(11.11%)	6(8.33%)	0.409
More than 5 mm	17(100%)	8(88.89%)	66(91.67%)	

Table 21: Comparison of PR expression with histological parameters

Comparison of ER and PR expression:

The ER and PR expression was compared with stage of the tumour, grade of the tumour, histological type, lymphovascular invasion and endometrial thickness.

Grade of the tumour:

62% tumours in category III (ER) were well differentiated and 93% of tumours in Category I (ER) were poorly differentiated. 40% of tumours in category III (PR) were well differentiated and 90% of tumours in Category I (PR) were poorly differentiated. The p value was <0.05 and is statistically significant.

Type of the lesion:

ER expression:

All the cases of disordered proliferation (100%) belonged to Category III. In typical hyperplasia 97% cases belonged to Category III. In atypical hyperplasia nineteen out of twenty cases (76%) belonged to Category III. In endometrioid carcinoma, 10% cases belong to Category I, 55% cases belonged to Category II and 35% cases belonged to Category III. In serous carcinoma three out of four cases (75%) belonged to Category I. In carcinosarcoma, 71% of cases belonged to Category I and 29% cases belonged to Category II. The p value is <0.05 and is statistically significant.

PR Expression:

All the cases of disordered proliferation (100%) and typical hyperplasia (100%) belonged to Category III. In atypical hyperplasia, twenty three out of twenty five cases (92%) belonged to Category III. In endometrioid carcinoma, 25% cases belong to Category

I, 30% cases belonged to Category II and 45% cases belonged to Category III. In serous carcinoma all the four cases belonged to Category I. In carcinosarcoma, 86% of cases belonged to Category I. The p value is <0.05 and is statistically significant.

Pathological correlation between curettings and resection specimens in preneoplastic lesions:

Out of the 21 cases of disordered proliferation in curettings, one case progressed to typical hyperplasia in resection specimen. Out of the 29 cases of typical hyperplasia in curettings, total six cases had a progression. Three cases progressed to atypical hyperplasia on resection. Out of the other three, two cases had a diagnosis of well differentiated endometrioid carcinoma and one was moderately differentiated in the resection specimen. Out of the 25 cases of atypical hyperplasia, eight cases had a progression. Out of the eight cases six cases had a diagnosis of well differentiated endometrioid carcinoma and two was moderately differentiated in the resection specimen.

DISCUSSION:

Endometrial carcinoma is one of the most common gynecological malignancy in developed countries. However, the incidence of endometrial carcinoma is increasing in developing countries due to increasing incidence of life style diseases such as obesity and diabetes (86). Various studies have previously assessed the expression of different markers in endometrial carcinoma to predict the outcome and prognosis, one of which is expression of hormonal receptors, ER and PR. This study analyses the clinico-pathological features of preneoplastic lesions and carcinoma of the endometrium along with immunohistochemical expression of ER and PR in both of the groups and compares it with the previously published studies.

Clinico-pathological features:

Age:

The age distribution of in this study population was 29-77 years. The mean age of presentation of preneoplastic lesions was 51 years. In preneoplastic lesions, the frequency of disordered proliferation was a decade earlier than other lesions. The mean age of carcinomas was 58 years. Endometrioid carcinoma in this study was seen a decade earlier than serous carcinoma and carcinosarcoma. This is in line with the fact that endometrial serous carcinoma and carcinosarcoma commonly occur in older age group (usually occur after 60yrs). The preneoplastic lesions in this study were seen in relatively younger patients compared to carcinoma. This was statistically significant ($p<0.05$). The transformation from preneoplastic to malignancy developed over a time period with progressive increase

in the degree of abnormality of the lining epithelium. According to Masjeed et al and Kumari et al, the peak incidence of endometrial hyperplasia (preneoplastic lesions) was in the fifth decade and endometrial carcinomas was in the sixth decade. (87,88) which was similar to this study.

Postmenopausal status:

In this study, 84% cases of carcinoma were post-menopausal, compared to 47% cases of preneoplastic lesions. This was statistically significant ($p<0.05$). According to Hileeto et al, preneoplastic lesions were more prevalent (42%) in the perimenopausal age group(89), which was similar to this study. As a result Chavez et al, proposed the role of minimally invasive techniques like hysteroscopy and sonohysterogram in the evaluation of menstrual irregularities in younger women as one of the reasons for earlier detection of preneoplastic lesions. (90)

Presenting complaints:

The most common clinical presentation (63%) in cases of carcinoma was post-menopausal bleeding. The most common presentation (52%) in preneoplastic lesions was abnormal uterine bleeding. This was statistically significant ($p<0.05$). This was in concordance with the study by Masjeed et al in which the most common clinical presentation was postmenopausal bleeding (52%), followed by menorrhagia (44%).

Risk factors of endometrial carcinoma:

In this study, 35% cases of preneoplastic lesions were overweight and 46% were obese. In cases of carcinoma, 30% were overweight and 32% were obese. This was similar to the study by Suskic et al wherein 45% of endometrioid

carcinoma had a BMI>30 kg/m2 (91), which was similar to this study. This was mainly due to the fact that in post-menopausal females, the main site of conversion of androstenedione to estrone is the adipose tissue (39). The incidence of carcinoma was 34% and 42% in parous and nulliparous women respectively in this study**.** According to Poccobelli et al, ever having birth was associated with a 35% reduced risk of developing endometrial cancer (odds ratio: 0.65) (92). This was in concordance with our study. In this study 39% and 8% had history of exogenous hormone intake in preneoplastic lesions and carcinoma respectively. Higher intake of exogenous hormones in preneoplastic cases was due to the fact these cases presented with menstrual irregularities at a relatively younger age. This was statistically significant ($p<0.05$)

Endometrial thickness:

In this study, the mean endometrial thickness (ET) in cases with preneoplastic lesions was 13.27mm. The mean endometrial thickness in cases of carcinomas was 26.13mm. This was statistically significant ($p<0.05$). In this study, 90% of patients with endometrial carcinoma had ET> 5mm. Various studies in the past(Gull et al and Ferrizzi et al) assessed the usefulness of ET in predicting the risk of cancer development in post-menopausal females (93,94). Meta-analysis of 5892 symptomatic women showed than an ET greater than or equal to 5mm was seen in 95% of endometrial cancers (95). This was similar to the present study.

Immunohistochemical expression of ER and PR:

ER and PR expression in endometrial preneoplastic lesions and carcinomas:

Combining category II and III in this study, ER was positive in 100% cases of disordered proliferation, 100% cases of typical hyperplasia, 96% of atypical hyperplasia. ER was positive in 90% of endometrioid carcinoma. PR was positive in 100% of disordered proliferation, 100% of typical hyperplasia, 96% of atypical hyperplasia. PR was positive in 75% of endometrioid carcinoma**.** None of the serous and carcinosarcoma cases were positive for ER and PR expression. This was statistically significant ($p<0.05$).

Various studies have previously assessed the expression of ER and PR in endometrial carcinoma and its preneoplastic lesions. The difference in the expression of ER and PR between this study and others is probably due to the different cut off value set for assessing ER and PR expression. The following table shows the expression of ER and PR in different studies(87,88,96). The findings were similar to this study.

Diagnosis	ER expression				
	Present study	Masjeed et al	Orejuela et al	Bozdogan et al	Kumara et al
Disordered proliferation	100 %	---	---	---	---
Typical hyperplasia	100 %	92.85 %	100 %	96.1 %	100 %
Atypical hyperplasia	96 %	85.71 %	---	---	---
Endometrioid carcinoma	90 %	60.71 %	71.4 %	86.3 %	68 %
Serous carcinoma	25 %	---	----	---	----
Carcinosarcoma	28 %	---	----	----	----

Table 22: Comparison of ER expression between present study and other studies

Diagnosis	PR expression				
	Present study	Masjeed et al	Orejuela et al	Bozdogan et al	Kumara et al
Disordered proliferation	100 %	---	---	---	---
Typical hyperplasia	100 %	90 %	94.7 %	100 %	100 %
Atypical hyperplasia	96 %	85.71 %	---	---	---
Endometrioid carcinoma	75 %	64.28 %	94.7 %	90.9 %	76 %
Serous carcinoma	25 %	---	----	---	----
Carcinosarcoma	14 %	---	----	----	----

Table 23: Comparison of PR expression between present study and other studies

Grade of the tumour:

In this study, among 20 cases of endometrioid carcinoma, 6 were well differentiated, 8 were moderately differentiated and 6 were poorly differentiated. 2 out of 20 cases (10%) of endometrioid carcinomas were negative for ER (one was moderately differentiated and other was poorly differentiated). None of the well differentiated endometrioid carcinomas were ER negative. 5 out of 20 cases (25%) of endometrioid carcinomas were negative for PR (four were poorly differentiated and the other one was moderately differentiated). This was statistically significant ($p<0.05$). The following tables

shows the expression of ER and PR in different grades of endometrioid carcinoma in the previous studies (95,96). Few of the recent studies done by Moriya et al, showed that the positive expression of PR correlates with the low grade of the tumour, low recurrence rate and higher survival in patients. The findings were similar to this study.

Grade	ER Expression		
	Present study	Srijaipracharoen et al	Kounelis et al
Well differentiated	100 %	77 %	88 %
Moderately differentiated	85 %		
Poorly differentiated	83 %	36.2 %	30.8 %

Table 24: Comparison of ER expression in grade of tumour between present study and other studies

Grade	PR Expression		
	Our study	Srijaipracharoen et al	Kounelis et al
Well differentiated	100 %	82 %	85.2 %
Moderately differentiated	85 %		
Poorly differentiated	33.3 %	44.7 %	46.2 %

Table 25: Comparison of PR expression in grade of tumour between present study and other studies

Stage of the tumour:

In this study, thirty cases (79%) had stage I disease, seven cases (18%) had stage III disease and one case (3%) had stage IV disease**.** Within endometrioid carcinoma, seventeen cases (85%) had stage I disease and two cases (10%) had stage III disease and one case (5%) had stage IV disease**.** Within serous carcinoma 2 cases (50%) had stage I disease and 2 cases (50%) had stage III disease**.** Eleven out of fourteen cases (79%) of carcinosarcoma had stage I disease and three cases (21%) had stage III disease. The ER expression in relation to the stage of the tumour is statistically insignificant ($p>0.05$)**.** The PR expression in relation to the stage of the tumour is statistically significant. (p value<0.05).

Stage	ER Expression					
	Present study	p value	Srijaipracharoen et al	p value	Kounelis et al	p value
Stage I	70 %	0.117	62.4 %	0.208	100%	0.0001
Stage II	----					
Stage III & IV	37 %		47.8 %		40%	

Table 26: Comparison of ER expression in stage of tumour between present study and other studies

Stage	PR Expression					
	Present study	p value	Srijaipracharoen et al	p value	Kounelis et al	p value
Stage I	56.66 %	0.045	69.4 %	0.122	100%	0.0006
Stage II	----					
Stage III & IV	12.5%		52.2 %		45%	

Table 27: Comparison of PR expression in stage of tumour between present study and other studies

Lymphovascular invasion was seen in 9 out of 38 (24%) cases of carcinoma. In endometrioid carcinoma, 3 out of 20 (15%) cases had lymphovascular invasion. None of the cases of serous carcinoma had lymphovascular invasion. In carcinosarcoma, 6 out of 14 (42.85%) cases had lymphovascular invasion. The expression of PR in patients with lymphovascular invasion was statistically significant with a p value of <0.05.

Lymphovascular invasion	Our study		Srijaipracharoen et al		Maniketh et al	
	ER +ve	ER -ve	ER +ve	ER -ve	ER +ve	ER -ve
Yes	33.33 %	66.67 %	45 %	55 %	83.3	16.7%
No	72.41 %	27.59 %	62.5 %	37.5 %	56.2%	43.8%

Table 28: Comparison of ER expression in relation to lymphovascular invasion between present study and other studies

Lymphovascular invasion	Our study		Srijaipracharoen et al		Maniketh et al	
	PR +ve	PR -ve	PR +ve	PR -ve	PR +ve	PR-ve
Yes	11.11 %	88.89 %	45 %	55 %	100%	0%
No	58.62 %	41.38 %	70.5 %	29.5 %	81.25%	18.75%

Table 29: Comparison of PR expression in relation to lymphovascular invasion between present study and other studies

In this study, there expression of ER and PR with depth of myometrial invasion, association with other benign lesions was not statistically significant.

CONCLUSION:

Precursor lesions:

1. The mean age of preneoplastic lesions of endometrium is 50 years.
2. History of exogenous hormone use is more frequently associated with preneoplastic lesions.

Carcinomas:

1. The mean age of carcinoma is 58 years.
2. Endometrioid carcinoma, occurred a decade earlier than serous and carcinosarcoma.
3. Endometrial carcinomas are more frequently associated with post-menopausal status.
4. Nulliparity is associated with relatively increased risk of carcinoma.
5. The mean endometrial thickness in patients with carcinomas is 26.13mm.
6. Carcinomas are associated with mean endometrial thickness of >5mm.

ER and PR expression:

1. There was no significant difference in the expression of ER and PR in the preneoplastic lesions.
2. Strong expression of ER and PR is seen in well and moderately differentiated endometrioid carcinoma compared to poorly differentiated endometrioid carcinomas, which are mostly negative.
3. All high grade serous carcinomas and carcinosarcoma are negative for ER and PR.

4. The difference in the immunohistochemical expression of ER and PR in endometrioid and serous carcinoma support the different pathogenesis of their development.
5. ER and PR expression decreases as the grade of the tumour increases.
6. PR expression decreases as the stage of the tumour increases.
7. There is no significant difference in ER expression with increasing stage of tumour.

LIMITATIONS:

1. Small sample size in individual groups.
2. The intensity of ER and PR expression is subjective to assess, especially between strong (2+) and very strong (3+).

FUTURE DIRECTIONS:

In association with other immunomarkers, p53 and PTEN, ER and PR can be used in predicting the biological behaviour and treatment in patients diagnosed with endometrial carcinoma.

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ANNEXURE - I

Procedure for immunohistochemistry:

Protocol for automated immunostaining:

1. Paraffin embedded tissue sections were cut at 4µ thickness and floated in poly L lysine coated slides incubated overnight at 37degree Celsius.

2. These slides were then treated with 4% milk solution for 10 minutes to eliminate the hydrophobic effect and give positive charge to the slides.

3. Then the slide labels were bar coded and the labelled slides were loaded in Ventana Benchmark XT autostainer (a fully automated autostainer).

4. Individual protocols has been divised in the software attached to the machine for each marker. Specific protocols were selected according to the marker.

5. A standard protocol was used for most of the markers with minimal variation for few individual markers. The steps included in the protocol were as follows.

a) Deparaffinisation

b) Liquid coverslip application

c) Heat induced antigen retrieval by treating with standard CC1 solution (ph patent for the company) for one hour at 90 degree Celsius.

6. Then the primary antibody was added and incubated for 40 min at 37 deg Celsius.

7. Then the secondary antibody was added and incubated for 8 minutes.

8. Finally the slides were counterstained with haematoxylin and incubated for 8 min, followed by incubation with bluing agent for 4 min.

9. From antigen retrieval till counterstaining, in between every step, the slides were washed with reaction buffer. The whole processing is automated. Then the slides were brought to 80% alcohol (2 changes) to remove the liquid coverslip and then dried and mounted in DPX.

Antibody	Clone	Dilution	Source
Estrogen receptor	SP1 Rabbit Monoclonal Antibody	Ready to use	Ventana
Progesterone receptor	1E2 Rabbit Monoclonal Antibody	Ready to use	Ventana

ANNEXURE - II

Proforma

Immunohistochemical evaluation of ER and PR in endometrial carcinoma and its precursors.

1. Serial No:

2. Biopsy No:

a) Curettings:

b) Resection:

3) Resection biopsy No (carcinoma)

4. Age:

5. Postmenopausal: 1=yes, 2=no

6. If YES, How many years?

CLINICAL FEATURES:

Complaints:

1 = Post-menopausal bleeding

2 = AUB

3= Abdominal pain

4=Others

BMI

2. History of OCP/HRT/Tamoxifen-1=yes, 2=no

3. Family history of other tumours: -1=yes, 2=no

If yes,

3. Syndromic association: -1=yes, 2=no,

If yes,

5. Comorbidities: 1= Hypothyroid

2=DM

3- HT

4=others

RADIOLOGICAL FEATURES:

ET: (mm)

GROSS FEATURES:

1. Pattern of growth: 1=Diffuse, 2=proliferative

2. Tumour size: (cm)

HISTOLOGICAL FEATURES:

1. Tumour differentiation:

1= Well differentiated, 2= Moderately differentiated, 3= Poorly differentiated.

2. Depth of invasion: mm

3. Myometrial invasion: 1= Less than half, 2= More than half: Y/N

4. Lymphovascular invasion: 1=yes, 2=no

5. Lymph node metastasis: 1=yes, 2=no

If yes Group of lymph node:

6. Stage: 1=1, 2 =2, 3=3, 4=4

7. Association with other lesions: 1 =Leiomyoma: 2=Endometriosis:

3=Adenomyosis:

8. Heterologous elements: 1=yes, 2=No

If yes _____

IMMUNOHISTOCHEMISTRY: (ER and PR)

The percentage of positive cells was graded as follows:

1 =Score 1- 0% to 25% of the nuclei

2= Score 2- 26% to 75% of nuclei stained

3= Score 3- more than 76% of the nuclei stained.

The staining intensity was scored as follows:

Score 1- absent or weak

Score 2- strong

Score 3- very strong.

1=Category I corresponded to a score of 2

2=Category II to a score of 3 or 4,

3=Category III to a score of 5 or 6.

Staining in adjacent stroma:1=yes, 2=No

IHC	%	Intensity	Category
ER			
PR			

ANNEXURE - III

IRB Documents

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

March 16, 2017

Dr Rima.S,
PG Registrar,
Department of General Pathology,
Christian Medical College,
Vellore - 632 004.

Sub: **Fluid Research Grant NEW PROPOSAL:**
Immunohistochemical evaluation of PTEN (phosphatase and tensin homolog) expression in endometrial biopsy samples of disordered proliferation, non atypical, & atypical hyperplasia and resection specimens of endometrial adenocarcinoma.
Rima.S, Employment Number: 33885, Post Graduate Registrar, Dr.Mayank Gupta, Employment Number:32630, Dr. Ramani Manoj Kumar. Employment Number: 10715 General Pathology. Mrs.Mahasampath Gowri.S, Employment No: 33418, Senior demonstrator, Dept. of Biostatistics,.

Ref: IRB Min No: 10436 [OBSERVE] dated 05.12.2016

Dear Dr Rima.S,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Biju George, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Dr. BIJU GEORGE
MBBS., MD., DM.,
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

Cc: Dr.Mayank Gupta, Dept. of General Pathology, CMC, Vellore

1 of 4

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Ref: IRB – A5 - 04.06.2018 July 13, 2018

Dr. Rima S,
Department of Pathology,
Christian Medical College,
Vellore 632 002

Ref: 1. IRB Min No: 10436 dated 05/12/2016

Dear Dr. Rima S,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed the following amendment for the study titled "Immunohistochemical evaluation of PTEN (phosphatase and tensin homolog) expression in endometrial biopsy samples of disordered proliferation, non atypical, & atypical hyperplasia and resection specimens of endometrial adenocarcinoma" on June 04th 2018.

(1) Change the Thesis Title from "Immunohistochemical evaluation of PTEN (phosphatase and tensin homolog) expression in endometrial biopsy samples of disordered proliferation, non atypical, & atypical hyperplasia and resection specimens of endometrial adenocarcinoma" to "Immunohistochemical evaluation of ER and PR in endometrial cancers and its precursors.

The following Institutional Review Board (Silver, Research & Ethics Committee) members were present at the meeting held on June 04th 2018 at 10.45 am in the New IRB Room, Christian Medical College, Bagayam, Vellore 632002.

Name	Qualification	Designation	Affiliation
Dr. Biju George	MBBS, MD, DM	Professor, Haematology, Research), Additional Vice Principal , Deputy Chairperson (Research Committee), Member Secretary (Ethics Committee), IRB, CMC, Vellore	Internal, Clinician
Rev. Joseph Devaraj	BSc, BD	Chaplaincy Department, CMC, Vellore	Internal, Social Scientist

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
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Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
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Chairperson, Ethics Committee.

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Dr. B. J. Prashantham	MA(Counseling Psychology), MA(Theology), Dr. Min (Clinical Counselling)	Chairperson, Ethics Committee, IRB. Director, Christian Counseling Centre, Vellore	External, Social Scientist
Dr. Anuradha Rose	MBBS, MD, MHSC (Bioethics)	Associate Professor, Community Health, CMC, Vellore	Internal, Clinician
Dr. Thomas V Paul	MBBS, MD, DNB, PhD	Professor,Endocrinology, CMC, Vellore	Internal, Clinician
Mr. C. Sampath	BSc, BL	Advocate, Vellore	External, Legal Expert
Dr. Jayaprakash Muliyil	BSc, MBBS, MD, MPH, Dr PH (Epid), DMHC	Retired Professor, CMC, Vellore	External, Scientist & Epidemiologist
Ms. Grace Rebekha	M.Sc., (Biostatistics)	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Mr. Samuel Abraham	MA, PGDBA, PGDPM, M. Phil, BL.	Sr. Legal Officer, CMC, Vellore	Internal, Legal Expert
Dr. Ratna Prabha	MBBS, MD (Pharma)	Associate Professor, Clinical Pharmacology, CMC, Vellore	Internal, Pharmacologist
Mrs. Pattabiraman	BSc, DSSA	Social Worker, Vellore	External, Lay Person
Mrs. Sheela Durai	MSc Nursing	Professor, Medical Surgical Nursing, CMC, Vellore	Internal, Nurse
Dr. Tunny Sebastian	P.hd., (Biostatistics)	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Mrs. Nirmala Margaret	MSc Nursing	Addl. Deputy Nursing Superintendent, College of Nursing, CMC,Vellore	Internal, Nurse
Dr. Sathish Kumar	MBBS, MD, DCH	Professor, Child Health, CMC, Vellore	Internal, Clinician
Dr. Barney Isaac	M.B.,B.S. D.N.B (Respiratory Diseases)	Associate Professor, Pulmonary Medicine, CMC, Vellore	Internal, Clinician

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Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Dr. John Antony Jude Prakash	MBBS, MD	Professor, Clinical Microbiology, CMC, Vellore.	Internal, Clinician.
Dr. Ajith Sivadasan	MD, DM	Professor, Neurological Sciences, CMC, Vellore	Internal, Clinician
Dr. Rekha Pai	BSc, MSc, PhD	Associate Professor, Pathology, CMC, Vellore	Internal, Basic Medical Scientist
Dr Sneha Varkki	MBBS, DCH, DNB	Professor, Paediatrics, CMC, Vellore	Internal, Clinician

We approve the above amendment as presented.

Yours sincerely,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board.

Dr. BIJU GEORGE
MBBS.,MD., DM.
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

IRB Min No: 10436 dated 05/12/2016

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ANNEXURE - IV

Data sheets.

Sl. No	C. No	Age (Years)	PM (Years)	Years	Gravida	BMI	Complaints	Others 1	OCP	H/o Tumor	Syndrome	Co-morbidities
1	23161/14	51	1	10	1	3	1	NA	2	2	2	3
2	46883/14	47	1	8	1	4	1	NA	2	1, Uterine cancer, Mother	2	2,3
3	1531/16	40	2	NA	1	3	2	NA	2	2	2	2
4	9381/16	41	2	NA	1	3	2	NA	1	2	2	4
5	36608/14	48	2	NA	1	2	2	NA	2	2	2	4
6	18773/16	43	2	NA	1	Not known	2,3	NA	2	2	2	2
7	28205/14	49	2	NA	1	3	2	NA	1	2	2	4
8	42025/16	46	2	NA	1	2	2	NA	1	2	2	4
9	44583/16	48	2	NA	1	3	2	NA	2	2	2	3
10	28085/16	47	2	NA	1	4	2	NA	2	2	2	1,2
11	44106/15	53	2	NA	1	2	2	NA	2	2	2	4,5
12	46037/15	46	2	NA	1	3	2	NA	1	1, Mother,Breast cancer	2	4
13	39269/16	42	2	NA	1	5	2	NA	1	2	2	4
14	42196/16	39	2	NA	1	4	2	NA	2	2	2	2
15	6802/16	52	2	NA	1	3	2	NA	1	1, CIN II (Self)	2	4
16	17849/14	46	2	NA	1	4	2	NA	2	2	2	4
17	36627/15	42	2	NA	1	3	2	NA	1	2	2	4
18	2546/14	46	2	NA	1	2	2	NA	1	2	2	4
19	41002/14	46	2	NA	1	3	2	NA	2	2	2	5
20	24960/14	41	2	NA	1	2	2	NA	1	2	2	2
21	28141/14	56	2	NA	1	2	2	NA	2	2	2	4
22	10946/15	46	2	NA	1	4	2	NA	1	1, Liver cancer, Father	2	4
23	16654/16	51	2	NA	2	3	2	NA	1	2	2	1,2
24	42108/16	60	1	12	1	3	1	NA	2	2	2	4
25	41844/16	52	1	10	1	5	1	NA	2	2	2	3,5
26	33707/16	61	1	12	1	6	1	NA	2	2	2	3
27	9540/16	63	1	12	1	5	1	NA				2,3
28	259/16	68	1	16	2	4	1	NA	2	2	2	4
29	33730/14	50	1	5	1	2	1	NA	1	1, Endometrial carcinoma, Mothe	2	2,5
30	857/16	70	1	20	1	2	1	NA	2	2	2	2,3
31	24980/14	50	1	2	1	4	1	NA	1	2	2	2,3
32	34645/14	58	1	3	1	5	1	NA	2	2	2	1,2,3,5
33	5912/14	55	1	4	1	6	1	NA	2	2	2	3
34	4604/16	53	1	10	1	5	1	NA	2	1, Uterine cancer, Mother	2	4
35	5264/14	39	2	NA	1	3	2	NA	1	2	2	5
36	2767/14	37	2	NA	1	4	2,4	Dysmennorhea	1	2	2	4
37	26934/14	52	1	2	1	4	1	NA	1	2	2	1
38	27872/15	49	2	NA	1	3	2	NA	2	2	2	4
39	6556/14	52	2	NA	1	2	2,4	Dysmennorhea	1	2	2	4
40	33938/14	62	1	10	2	6	1	NA	2	2	2	3,5
41	14235/15	52	1	5	1	5	1	NA	1	2	2	1,2,5
42	4870/14	53	2	NA	1	3	2	NA	2	2	2	4
43	8741/16	61	1	16	1	4	1	NA	2	1, Gall bladder carcinoma, Mothe	2	4
44	4534/14	51	1	1.5	1	3	1	NA	2	2	2	2,3
45	39849/16	44	2	NA	1	3	2	NA	2	2	2	4
46	2540/14	43	2	NA	1	3	2	NA	1	2	2	4
47	22344/14	52	1	5	1	4	1,3	NA	2	2	2	4
48	36807/15	52	1	5	1	4	1	NA	2	2	2	4
49	38146/15	54	1	8	1	6	1	NA	2	2	2	3
50	26977/14	48	1	Not known	Not known	Not known	1	NA	2	2	2	2
51	16810/15	50	2	NA	1	1	2	NA	1	2	2	2,5
52	23519/14	60	1	10	2	3	1	NA	2	2	2	1,2,3
53	13404/15	45	2	NA	1	3	2	NA	1	2	2	4
54	190/14	70	1	30	1	3	1	NA	2	2	2	3,5
55	45417/15	64	1	10	1	6	1	NA	2	2	2	2,3,5
56	43437/15	33	2	NA	1	4	2	NA	1	2	2	4
57	13810/16	57	1	8	1	6	1	NA	2	2	2	3
58	48570/14	74	1	24	Not known	Not known	1	NA	2	2	2	2,3,5
59	11733/15	54	1	2	1	4	1	NA	2	2	2	3
60	6926/15	48	2	NA	1	Not known	2	NA	2	2	2	2
61	32549/14	49	2	NA	2	4	2	NA	1	2	2	4
62	37061/16	29	2	NA	2	4	4	Primary Infertilty	1	2	2	4
63	45126/15	60	1	10	1	4	1	NA	1, Tamoxifen	1, Breast cancer (Self)	2	4
64	7599/14	52	1	3	1	3	1	NA	2	2	2	4
65	48105/14	29	2	NA	1	4	3	NA	1	2	2	4
66	33805/15	52	2	NA	1	2	2	NA	2	2	2	2
67	10725/15	48	2	NA	Not known	Not known	2	NA	Not known	2	2	4
68	16340/14	41	2	NA	Not known	Not known	2	NA	Not known	2	2	2,3
69	40571/15	60	1	9	2	4	1	NA	1	2	2	5
70	11096/16	59	1	4	1	3	1	NA	1	2	2	4
71	32979/14	50	1	1.5	1	3	2	NA	2	2	2	2
72	4035/14	62	1	8	1	6	1,4	White discharge	2	1, Stomach carcinoma (Self)	2	2,3
73	28403/16	51	2	NA	1	2	2,4	Vulval itching	2	2	2	5
74	46257/15	45	2	NA	1	2	2	NA	2	2	2	4
75	31744/14	70	1	30	1	3	1	NA	2	2	2	3,5

Others 2	ET (mm)	ER Percentage	ER Intensity	ER category	PR Percentage	PR Intensity	PR Category	Diagnosis	Resection	Resection-Diagnosis
NA	5	3	2	3	3	2	3	DPEM	27138/14	Basal
NA	8	3	2	3	3	2	3	DPEM	35754/15	Basal
NA	51	3	2	3	3	2	3	DPEM	98851/16	H.No atypia
NA	19	3	2	3	3	2	3	DPEM	15043/16	Progesterone effect
Na	10	3	2	3	3	2	3	DPEM	41719/14	Proliferative
NA	Not known	3	2	3	3	2	3	DPEM	20295/16	Late Proliferative
NA	17	3	2	3	3	2	3	DPEM	46240/14	Secretory
NA	10	3	2	3	3	2	3	DPEM	13554/17	Basal
NA	12	3	2	3	3	2	3	DPEM	25185/17	Proliferative
NA	6.2	3	2	3	3	2	3	DPEM	36565/16	DPEM
CAD	Not Known	3	2	3	3	3	3	DPEM	350/16	Proliferative
NA	11	3	3	3	3	3	3	DPEM	15913/16	Secretory
NA	32	3	2	3	3	3	3	DPEM	3370/17	Progesterone effect
NA	39	3	2	3	3	2	3	DPEM	44741/16	Secretory
NA	8	3	2	3	3	2	3	DPEM	15459/16	Basal
NA	7	3	2	3	3	3	3	DPEM	16148/15	Basal
NA	7	3	3	3	3	3	3	DPEM	41873/15	Proliferative
NA	15	3	2	3	3	3	3	DPEM	9840/14	Secretory
TB	Not known	3	2	3	3	2	3	DPEM	47639/14	Proliferative
NA	14	3	2	3	3	2	3	DPEM	31886/14	Progesterone effect
NA	12	3	2	3	3	2	3	DPEM	30380/14	DPEM
NA	6	3	2	3	3	3	3	H.No atypia	14674/15	Progesterone effect
NA	11	3	2	3	3	2	3	H.No atypia	258/17	Endometrial Polyp
NA	8	3	3	3	3	3	3	H.No atypia	16945/17	H. atypia
Dyslipidemia, OSA	10	3	2	3	3	2	3	H.No atypia	23970/17	Basal
NA	15	3	2	3	3	3	3	H.No atypia	37669/16	H. atypia
NA	7	3	3	3	3	3	3	H.No atypia	Not available	Not available
NA	14	3	3	3	3	3	3	H.No atypia	6058/16	H. No atypia
Dyslipidemia	7	3	2	3	3	2	3	H.No atypia	36055/14	Basal
NA	Not known	3	2	3	3	2	3	H.No atypia	7166/16	Endometrial Polyp
NA	5.6	3	2	3	3	2	3	H.No atypia	Not available	Not available
Dyslipidemia	12	3	2	3	3	2	3	H.No atypia	2235/15	H. No atypia
NA	10	3	3	3	3	3	3	H.No atypia	22289/16	Endometrial carcinoma
NA	13	3	3	3	3	3	3	H.No atypia	5862/16	Endometrial Polyp
Asthma	6	3	2	3	3	2	3	H.No atypia	26483/14	Proliferative
NA	6	3	2	3	3	2	3	H.No atypia	11667/14	Proliferative
NA	9.4	3	2	3	3	2	3	H.No atypia	Not available	Not available
NA	10	3	2	3	3	2	3	H.No atypia	33767/15	H. No atypia
NA	17	3	2	3	3	2	3	H.No atypia	15356/14	Basal
Dyslipidemia	Not known	3	2	3	3	2	3	H.No atypia	44616/14	Endometrial carcinoma
CAD	13	3	2	3	3	2	3	H.No atypia	2176/16	Progesterone effect
NA	16	3	2	3	3	2	3	H.No atypia	19773/14	H.No atypia
NA	10	3	2	3	3	2	3	H.No atypia	9981/16	H.No atypia
NA	Not known	3	2	3	3	2	3	H.No atypia	8469/14	Non secretory endometrium
NA	25	3	2	3	3	2	3	H.No atypia	3086/17	Basal
NA	25	3	2	3	3	2	3	H.No atypia	7124/14	Progesterone effect
NA	4	3	2	3	3	2	3	H.No atypia	27502/14	H.No atypia
NA	15	3	1	2	3	2	3	H.No atypia	45732/15	H.No atypia
NA	11	3	2	3	3	2	3	H.No atypia	Not available	Not available
NA	Not known	3	2	3	3	2	3	H.No atypia	Not available	Not available
Dyslipidemia	Not known	3	2	3	3	2	3	H.Atypia	Not available	Not available
NA	9	3	2	3	3	2	3	H.Atypia	24198/14	Endometrial carcinoma
NA	17	3	2	3	3	2	3	H.Atypia	19223/15	Proliferative
Dyslipidemia, CAD	5	3	2	3	3	2	3	H.Atypia	Not available	Not available
Asthma	12	3	2	3	3	2	3	H.Atypia	2834/16	H.No atypia
NA	13	3	2	3	3	2	3	H.Atypia	839/16	Progesterone effect
NA	10	3	2	3	3	2	3	H.Atypia	22289/16	Endometrial carcinoma
CLD	29	3	1	2	3	2	3	H.Atypia	Not available	Not available
NA	10	1	1	1	1	1	1	H.Atypia	Not available	Not available
NA	7	3	2	3	3	2	3	H.Atypia	Not available	Not available
NA	11	3	2	3	3	2	3	H.Atypia	Not available	Not available
NA	14	3	2	3	3	2	3	H.Atypia	17185/17	Endometrial carcinoma
NA	1.8	3	2	3	3	2	3	H.Atypia	Not available	Not available
NA	8	3	2	3	3	2	3	H.Atypia	Not available	Not available
NA	13	3	2	3	3	2	3	H.Atypia	19291/15	Progesterone effect
NA	13	3	1	2	3	1	2	H.Atypia	35586/15	Basal
NA	27	3	1	2	3	2	3	H.Atypia	Not available	Not available
NA	Not known	3	1	2	3	2	3	H.Atypia	Not available	Not available
Seizures	53	3	2	3	3	2	3	H.Atypia	48438/15	Basal
NA	6	3	2	3	3	2	3	H.Atypia	Not available	Not available
NA	9	3	1	2	3	2	3	H.Atypia	39184/14	Endometrial carcinoma
NA	13	3	2	3	3	2	3	H.Atypia	8867/14	H.Atypia
Schizophernia	9	3	2	3	3	2	3	H.Atypia	31987/16	H.Atypia
NA	12	3	2	3	3	2	3	H.Atypia	Not available	Not available
CAD	5	3	2	3	3	2	3	H.Atypia	Not available	Not available

Sl. No	C. No	Age (Years)	PM (Years)	Years	Gravida	BMI	Complaints	Others 1	OCP	H/o Tumor	Syndrome	Co-morbidities	Others 2	ET (mm)	Gross
1	272/14	39	2	NA	2	5	4	Primary Infertility	2	2	2	2,3	NA	9.3	2
2	9828/15	49	2	NA	1	5	2	NA	1	1, Grandma, Uterine cancer	2	3	NA	Not known	2
3	16926/14	62	1	18	1	5	1	NA	2	1, Mother, Uterine cancer	2	2,3	NA	17.7	2
4	11271/14	46	2	NA	1	2	2	NA	2	1, Mother, Endometrial cancer	2	5	Seizures	11	1
5	10821/14	59	1	4	1	5	2	NA	2	2	2	4	NA	8	2
6	11879/16	58	1	2	1	4	1	NA	2	2	2	2,3	NA	Not known	1
7	22218/14	68	1	10	1	4	1	NA	2	2	2	4	NA	79	1
8	15881/15	62	1	12	1	2	1	NA	2	2	2	2,3	NA	19	2
9	31229/14	60	1	10	1	4	1	NA	2	2	2	1,2,3	NA	10	2
10	42961/15	66	1	20	1	4	1	NA	2	2	2	2,3	NA	16	2
11	7294/15	51	1	5	1	2	3,4	White discharge	2	2	2	4	NA	57	2
12	43009/15	60	1	20	1	Not Known	1	NA	2	2	2	3,5	Dyslipidemia	Not known	2
13	25373/16	56	1	8	1	6	1	NA	2	2	2	3	NA	16	2
14	19431/16	67	1	23	1	6	1	NA	2	2	2	3	NA	3.4	2
15	21716/16	34	2	NA	1	2	2,3	NA	1	2	2	2,5	Dyslipidemia	7.2	2
16	31828/16	70	1	20	1	2	1	NA	2	1, Sister, Breast cancer	2	3	NA	2.5	2
17	5069/16	54	1	3	1	2	1,4	Urinary retention	2	2	2	1,2	NA	46	2
18	29085/16	71	1	30	1	2	1	NA	2	2	2	1	NA	5.4	2
19	12212/14	55	1	14	1	1	4	White discharge	2	1, Self, Spindle cell tumor	2	4	NA	12	2
20	12834/14	48	2	NA	2	2	4	Abdominal distension	2	2	2	1	NA	Not known	2
21	16409/14	60	1	18	1	3	1	NA	2	2	2	1,2,3	NA	18	2
22	10351/14	50	1	1.5	1	2	1	NA	2	2	2	3	NA	13.6	2
23	36549/15	57	1	10	1	3	1,3	NA	2	2	2	2,3	NA	12	2
24	33205/16	55	1	10	1	3	1	NA	2	2	2	3	NA	Not known	1
25	4811/15	77	1	25	1	3	1	NA	2	2	2	2,3	NA	44	2
26	7055/16	55	1	4	1	3	1	NA	2	2	2	1	NA	78	2
27	28249/15	65	1	20	1	3	1	NA	2	2	2	2,3	NA	40	2
28	25048/15	60	1	16	1	4	4	White discharge	1	2	2	2,3	NA	43	2
29	10073/14	75	1	20	1	3	4	Urinary retention	2	1, Self, Breast cancer	2	2,3	NA	37	2
30	9106/16	60	1	8	1	3	1	NA	2	2	2	2,3	NA	Not known	2
31	22048/14	62	1	7	1	3	4	White discharge	2	2	2	4	NA	38	3
32	32232/16	56	1	16	2	2	2	NA	2	2	2	3	NA	32	2
33	13184/16	56	1	3.5	2	3	1,4	White discharge	2	2	2	4	NA	14	1
34	11471/15	49	2	NA	2	1	2,3	NA	2	2	2	4	NA	10	2
35	28256/15	65	1	10	1	2	2	NA	2	2	2	3	NA	62	2
36	17439/14	60	1	15	1	2	1	NA	2	1, Sister, Uterine cancer	2	4	NA	19	1
37	38577/15	49	1	5	1	3	1	NA	2	2	2	2,3,5	CAD	15	2
38	2673/16	62	1	20	1	4	1	NA	2	2	2	4	NA	41	2

Tumor size (cm)	Grade	Depth of Invasion (mm)	Myometrium	Lymphovasuclar Invasion	Lymphnode mets	Group	TNM	Stage	Association	Heterologous	H. Elememts	ER Percentage	ER Intensity	ER category	PR Percentage	PR Intensity	PR Category	Diagnosis
5.5	1	3	1	2	2	NA	pT1a	1	5	2	NA	3	2	3	2	1	2	Endometrial carcinoma
11.5	3	11	2	2	2	NA	pT1b	1	1	2	NA	2	1	2	1	1	1	Endometrial carcinoma
4.6	2	6	1	2	2	NA	pT1a	1	5	2	NA	3	1	2	3	1	2	Endometrial carcinoma
4.2	3	4	1	2	2	NA	pT1a	1	5	2	NA	3	2	3	3	2	3	Endometrial carcinoma
1	1	3	1	2	2	NA	pT1a	1	5	2	NA	3	2	3	3	2	3	Endometrial carcinoma
2.5	2	3	1	2	2	NA	pT1a	1	5	2	NA	2	2	2	3	2	3	Endometrial carcinoma
4	2	11	2	1	1	Right pelvic & para ao	pT1bN2	3	3	2	NA	3	1	2	2	2	2	Endometrial carcinoma
4	2	1	1	2	2	NA	pT1a	1	5	2	NA	2	1	2	1	1	1	Endometrial carcinoma
3.6	2	6	1	2	2	NA	pT1a	1	3	2	NA	3	1	2	3	2	3	Endometrial carcinoma
2.3	2	2	1	2	2	NA	pT1a	1	1	2	NA	3	1	2	3	2	3	Endometrial carcinoma
5	1	8	1	2	2	NA	pT1a	1	1	2	NA	3	2	3	3	2	3	Endometrial carcinoma
0.6	1	2	1	2	Not known	NA	pT1a	1	5	2	NA	3	2	3	3	2	3	Endometrial carcinoma
3.9	2	12	2	2	Not known	NA	pT1b	1	5	2	NA	1	1	1	2	1	2	Endometrial carcinoma
8	3	11	2	2	2	NA	pT1b	1	1	2	NA	2	1	2	2	1	2	Endometrial carcinoma
5.5	3	3	1	2	1	Left pelvic	pT1aN1	3	5	2	NA	2	1	2	1	1	1	Endometrial carcinoma
4.5	1	2	1	2	2	NA	pT1a	1	5	2	NA	3	2	3	3	2	3	Endometrial carcinoma
3.5	1	4	1	2	2	NA	pT1a	1	1	2	NA	3	1	2	3	1	2	Endometrial carcinoma
4	2	15	2	2	2	NA	pT1b	1	5	2	NA	3	2	3	3	2	3	Endometrial carcinoma
4.7	3	11	2	1	2	NA	pT1b	1	5	2	NA	2	1	2	1	1	1	Endometrial carcinoma
10	3	40	2	1	1	Left pelvic & Mesentr	pT4N1	4	1	2	NA	1	1	1	1	1	1	Endometrial carcinoma
5	2	11	2	2	2	NA	pT1b	1	5	2	NA	3	2	3	3	2	3	Endometrial carcinoma & 20 - 30 % serous
1.5	3	3	1	2	Not known	NA	pT1a	1	1	2	NA	1	1	1	1	1	1	Serous
0.2	2	1	1	2	1	Left pelvic	pT1aN1	3	1,3	2	NA	2	1	2	1	1	1	Serous
2.5	3	15	2	2	Not known	NA	pT3a	3	5	2	NA	1	1	1	1	1	1	Serous
5	3	16	2	2	Not known	NA	pT1b	1	4, Benign se	2	NA	2	1	2	1	1	1	Carcino sarcoma
8.5	3	6	1	2	2	NA	pT1a	1	5	1	Cartilagenous	1	1	1	1	1	1	Carcino sarcoma
10.5	3	5	1	1	2	NA	pT1a	1	1	1	Osteocartilage	2	1	2	1	1	1	Carcino sarcoma
7.2	3	8	2	2	2	NA	pT1b	1	3	1	Rhabdomyosar	1	1	1	1	1	1	Carcino sarcoma
6	3	1	1	2	Not known	NA	pT1a	1	5	2	NA	1	1	1	1	1	1	Carcino sarcoma
6	3	7	2	1	2	NA	pT3a	3	5	1	Rhabdomyosar	1	1	1	1	1	1	Carcino sarcoma
10	3	18	2	1	1	right & Left pelvic, Rig	pT3aN2	3	5	1	Chondrosarcon	1	1	1	1	1	1	Carcino sarcoma
2.6	3	1	1	2	2	NA	pT1a	1	5	2	NA	2	1	2	1	1	1	Carcino sarcoma
3	3	5	1	2	2	NA	pT1a	1	5	2	NA	1	1	1	2	1	2	Carcino sarcoma
4.5	3	24	2	1	Not known	NA	pT1b	1	4, SCC cervi	2	NA	1	1	1	1	1	1	Carcino sarcoma
6	3	14	2	2	2	NA	pT1b	1	5	2	NA	1	1	1	1	1	1	Carcino sarcoma
4.5	3	11	2	1	1	right & Left pelvic, Rig	pT3aN2	3	5	2	NA	1	1	1	1	1	1	Carcino sarcoma
5	3	5	1	1	2	NA	pT1a	1	5	2	NA	1	1	1	1	1	1	Carcino sarcoma
3	3	1	1	2	2	NA	pT1a	1	5	1	Chondrosarcon	2	1	2	2	1	2	Carcino sarcoma

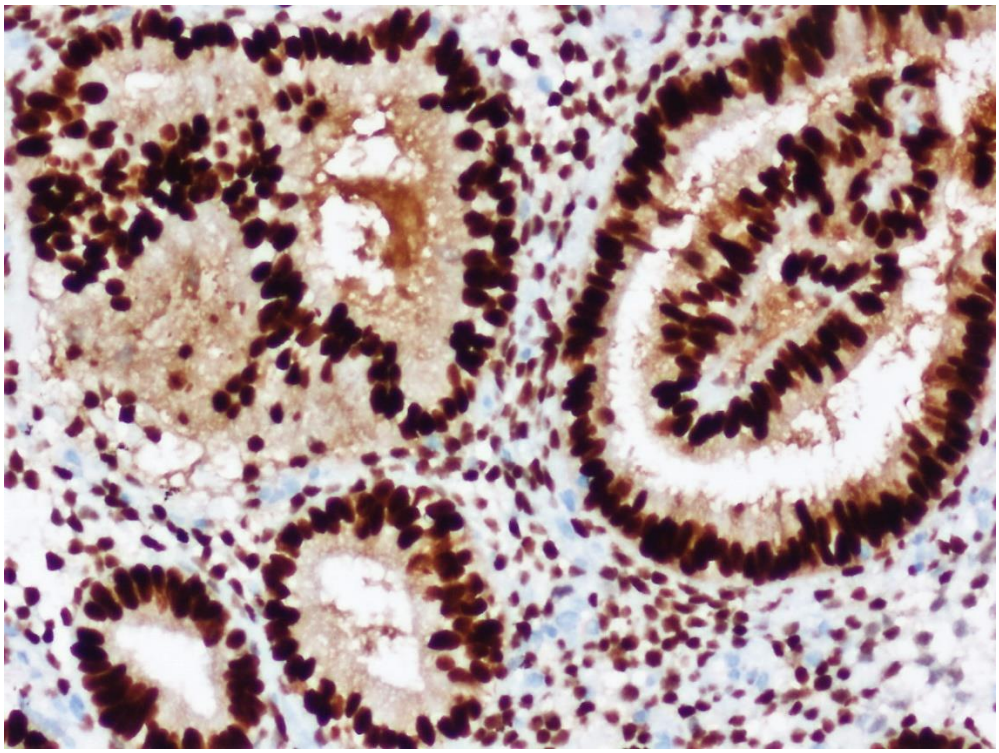

Figure 30: Proliferative endometrium with very strong ER expression (x200)

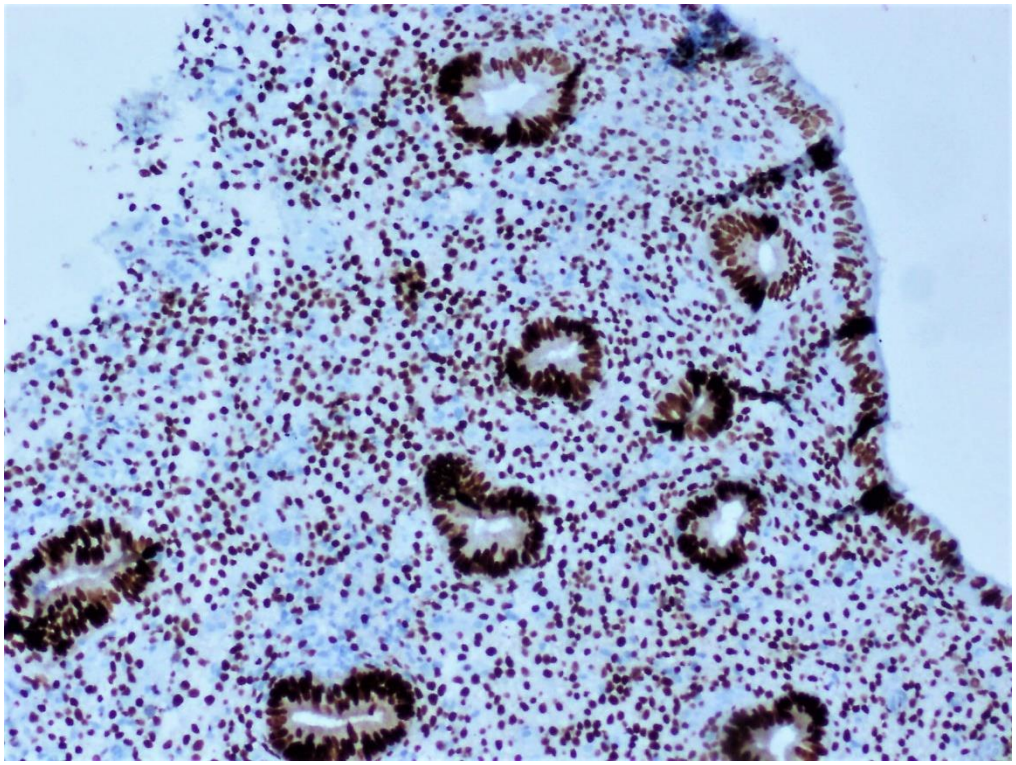

Figure 31: Proliferative endometrium with strong and very strong PR expression (x100)

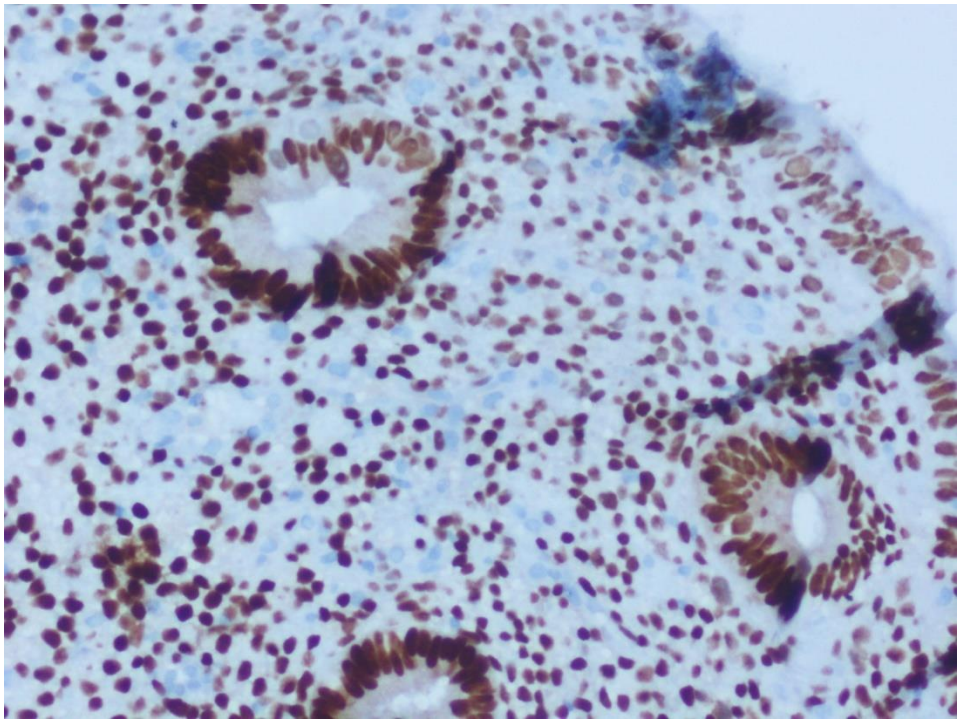

Figure 32: Proliferative endometrium with strong and very strong PR expression (x200)

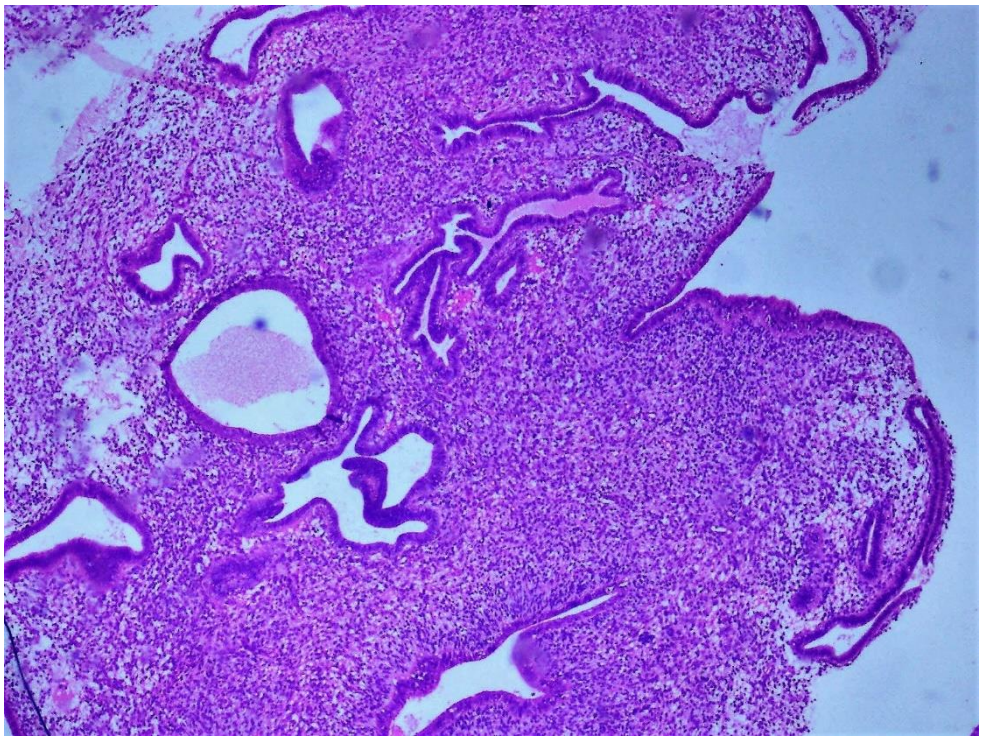

Figure 33: Disordered proliferation (H&E x40)

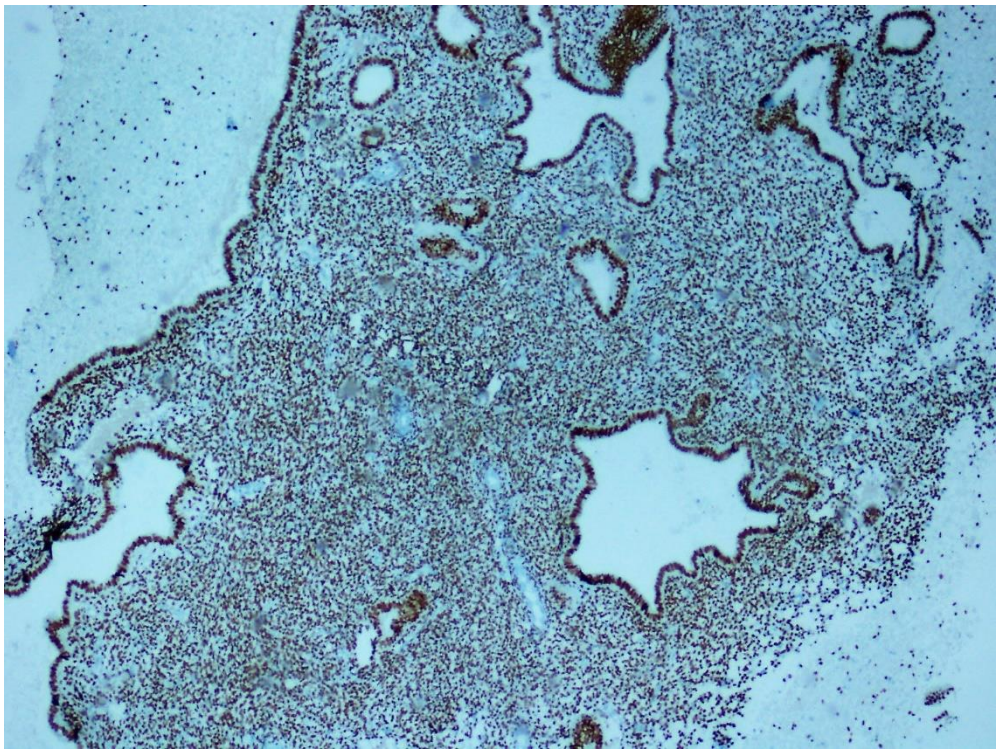

Figure 34: Disordered proliferation with strong and very strong ER expression (x40)

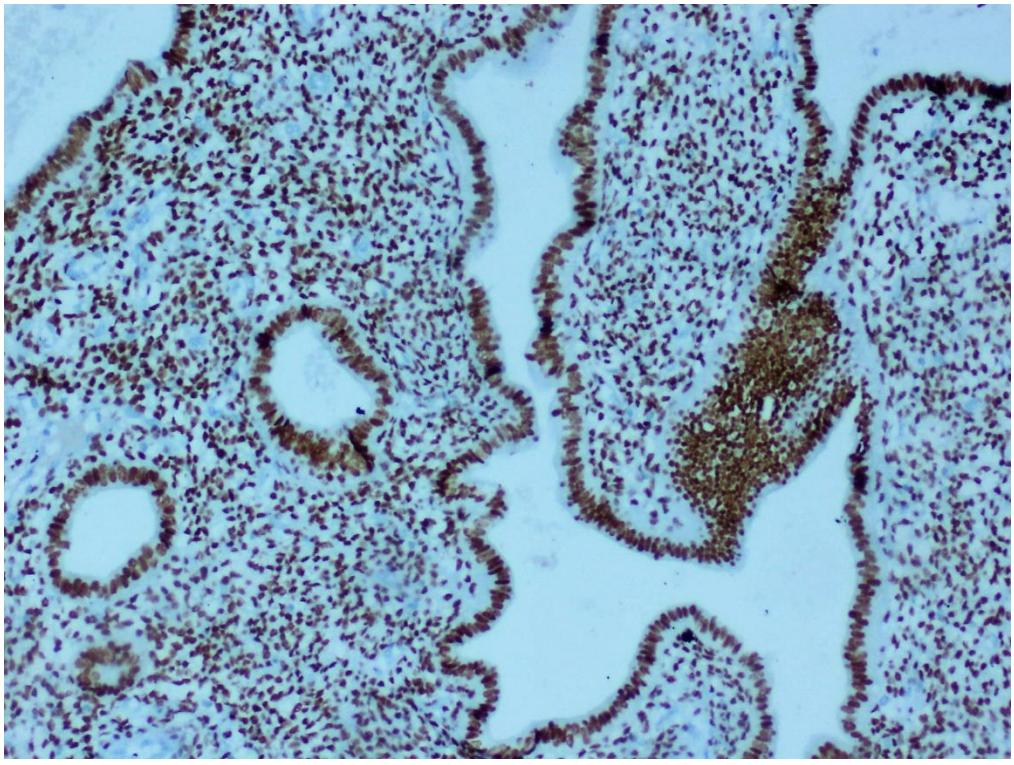

Figure 35: Disordered proliferation with strong and very strong PR expression (x100)

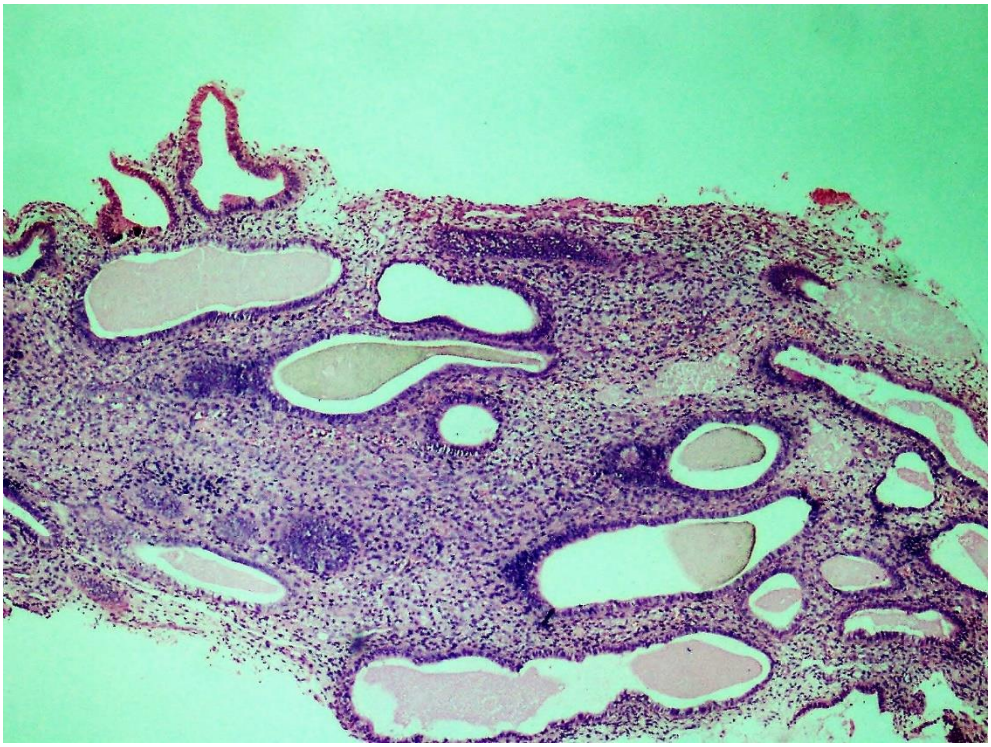

Figure 36: Typical hyperplasia (H&E x40)

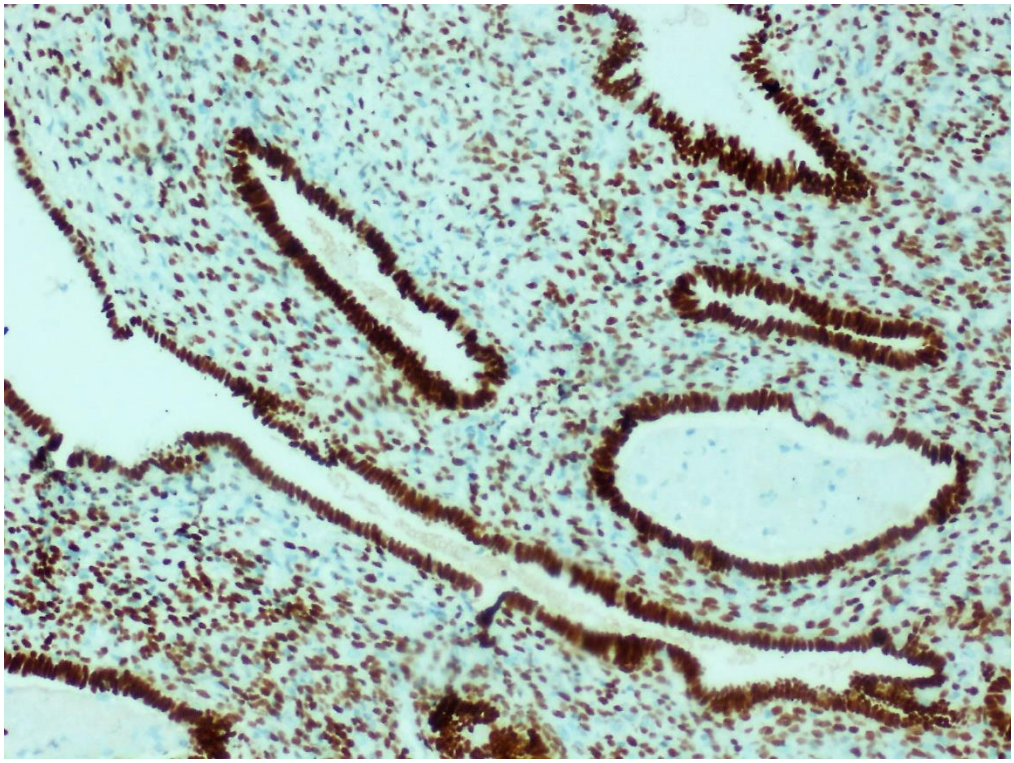

Figure 37: Typical hyperplasia with strong and very strong ER expression (x200)

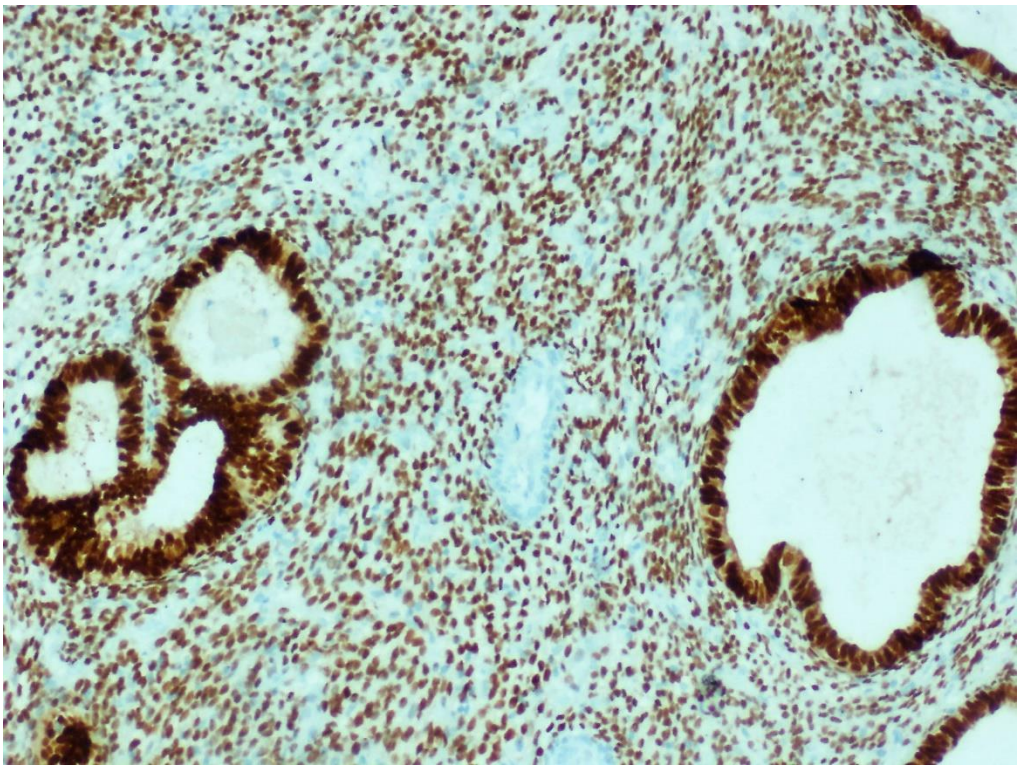

Figure 38: Typical hyperplasia with strong and very strong PR expression (x200)

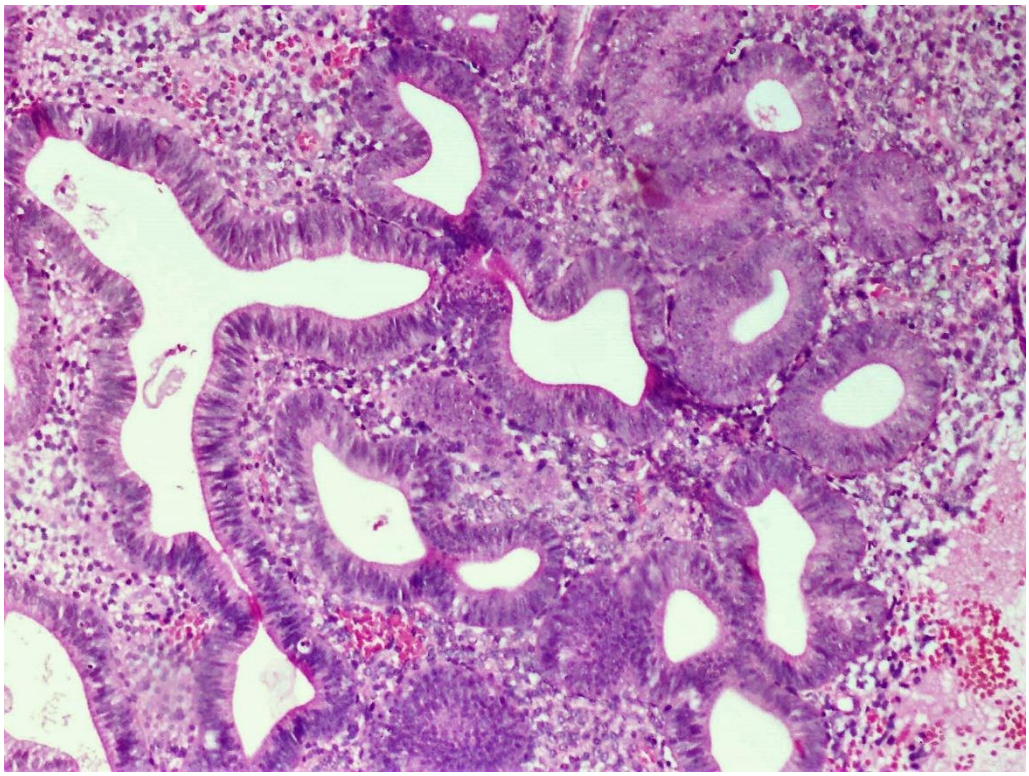

Figure 39: Atypical hyperplasia (H&E x200)

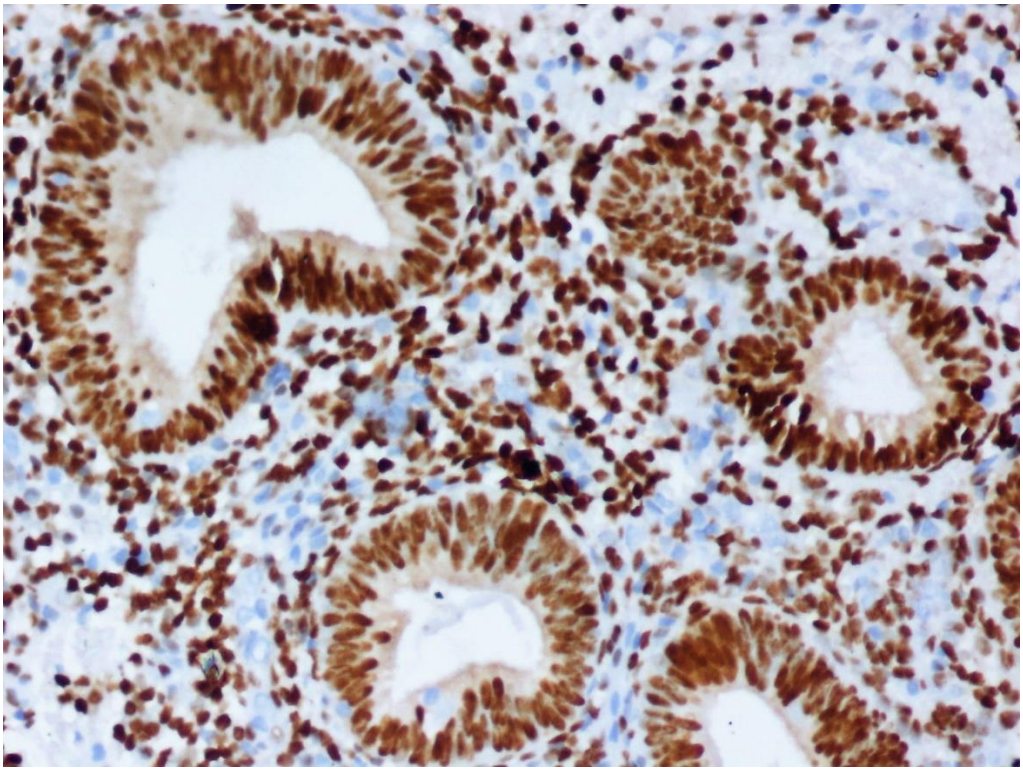

Figure 40: Atypical hyperplasia with strong and very strong ER expression (x200)

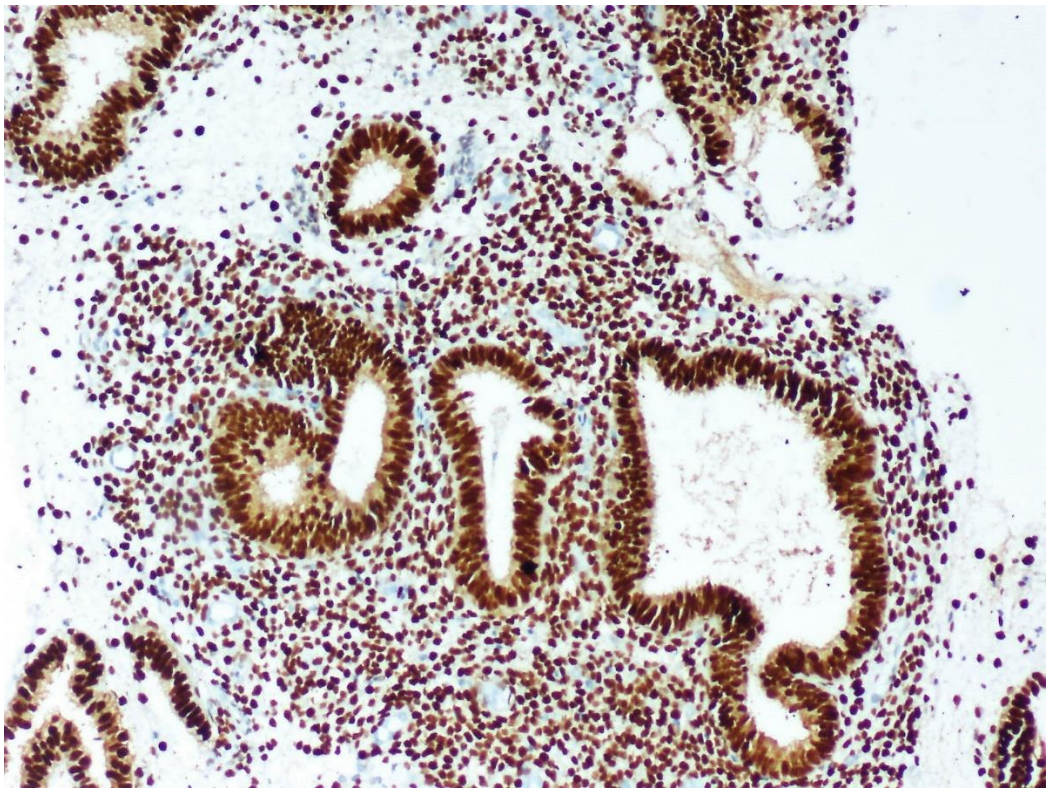

Figure 41: Atypical hyperplasia with strong and very strong PR expression (x200)

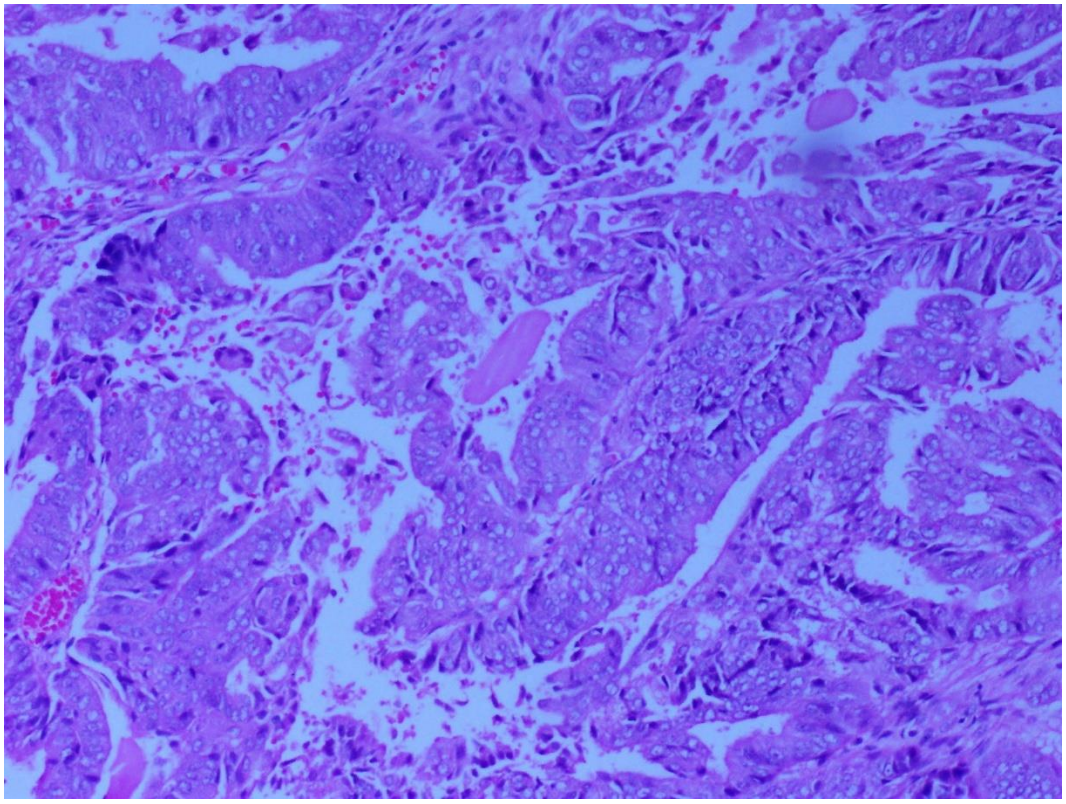

Figure 42: Well differentiated (Grade 1) endometrioid carcinoma (H&E x200)

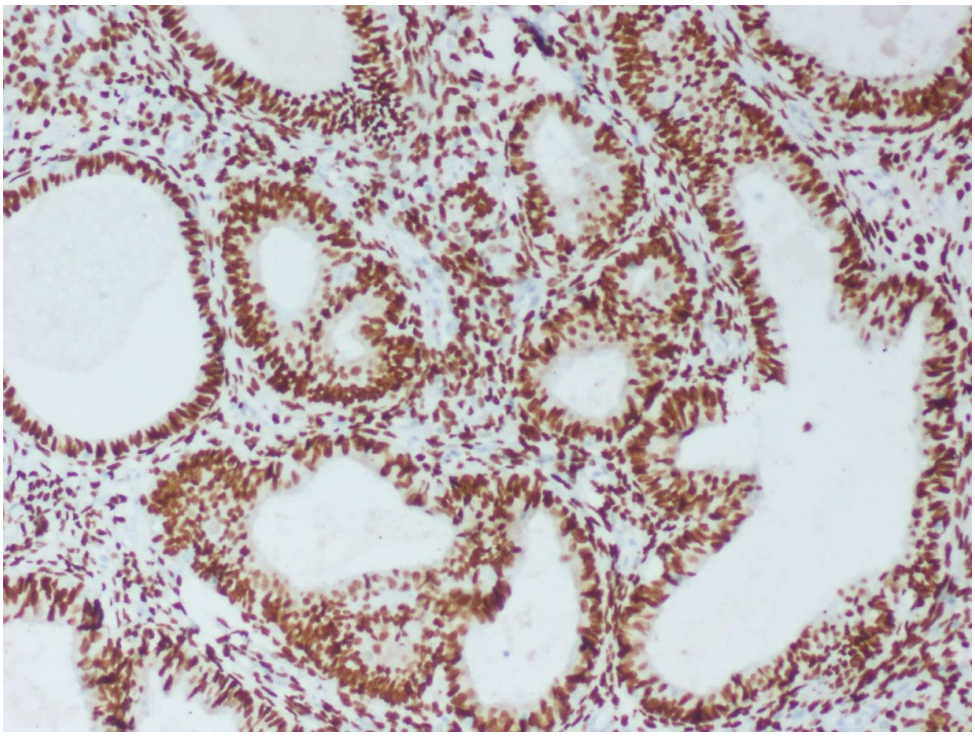

Figure 43: Well differentiated (Grade 1) endometrioid carcinoma with strong and very strong ER expression (x200)

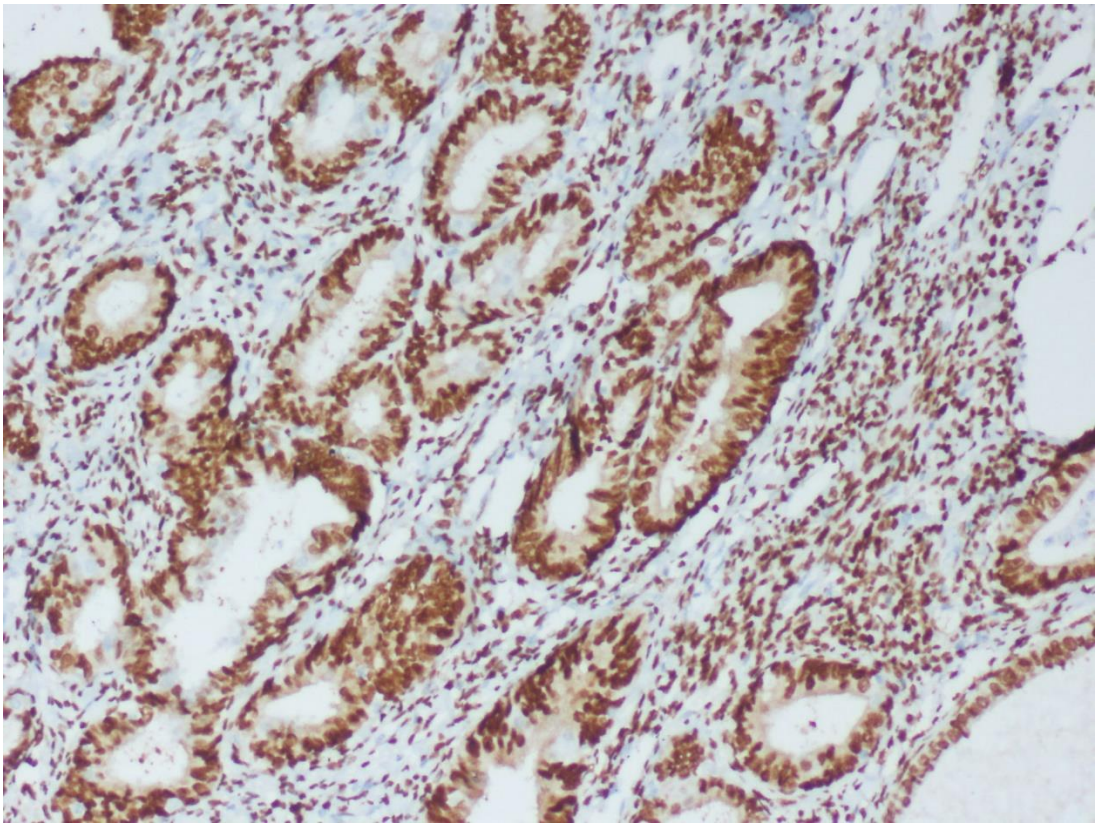

Figure 44: Well differentiated (Grade 1) endometrioid carcinoma with strong and very strong PR expression (x200)

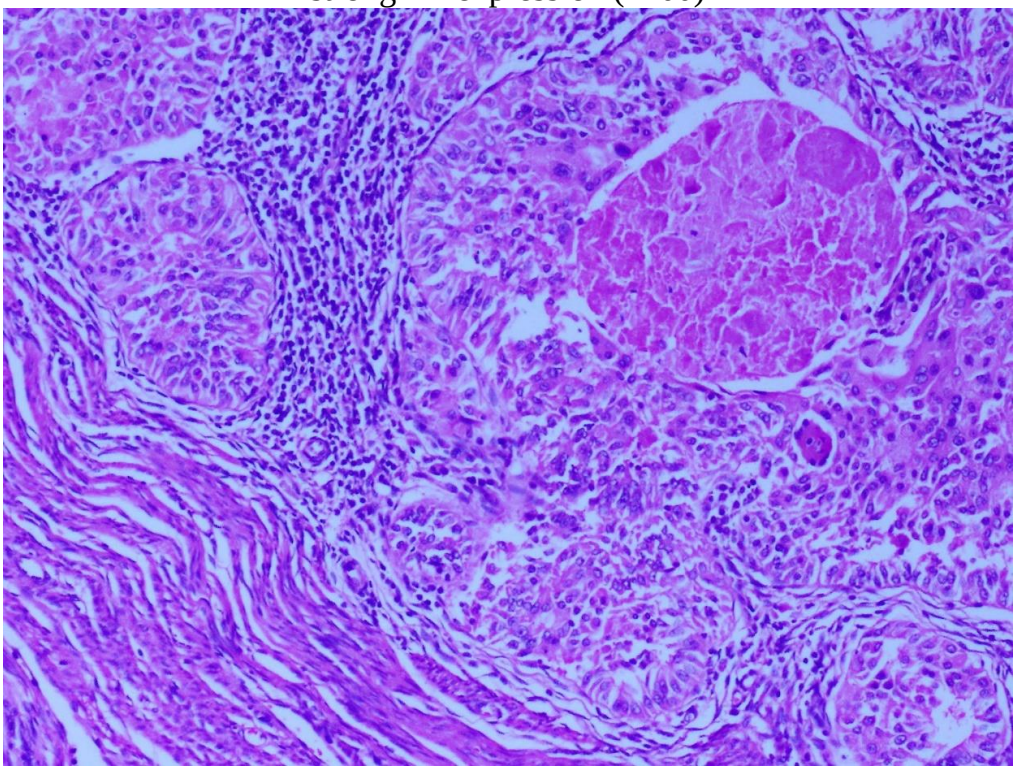

Figure 45: Poorly differentiated (Grade 3) endometrioid carcinoma (H&E x200)

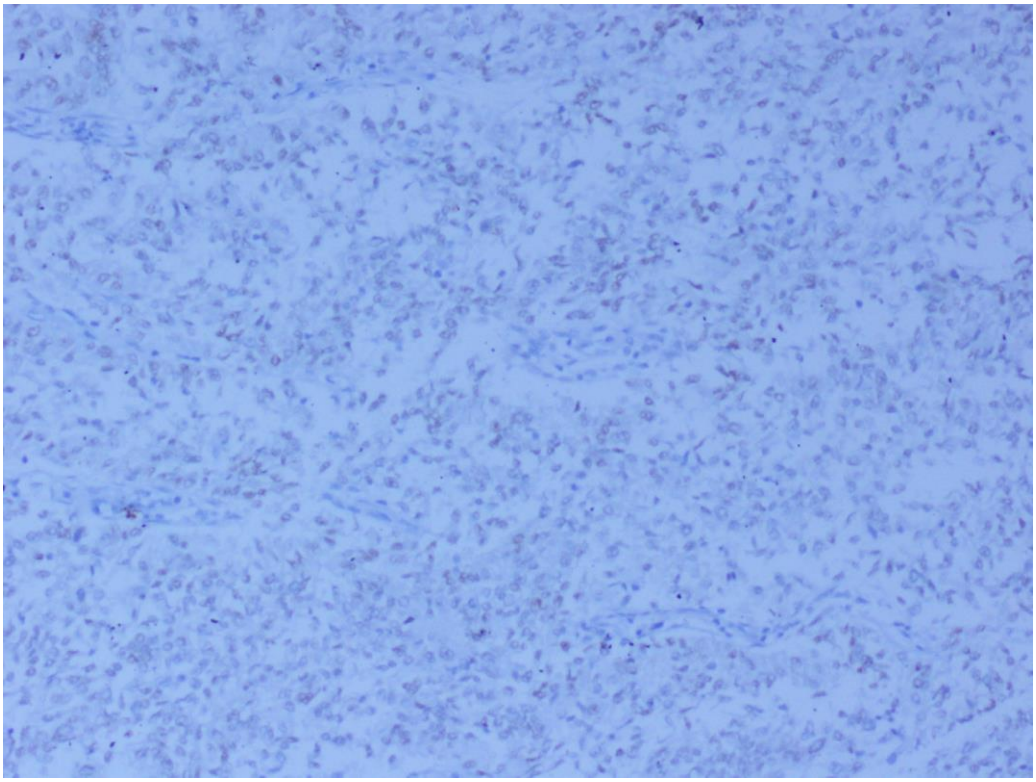

Figure 46: Poorly differentiated (Grade 3) endometrioid carcinoma with absent and weak ER expression (x100)

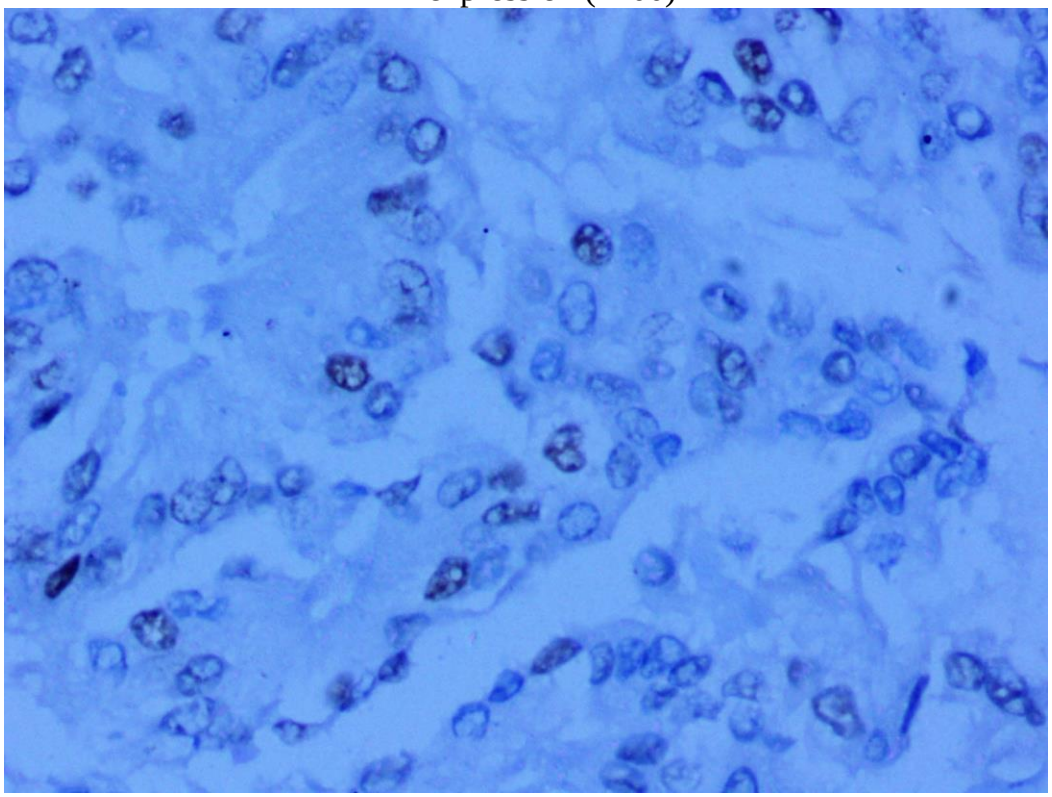

Figure 47: Poorly differentiated (Grade 3) endometrioid carcinoma with absent and weak ER expression (x400)

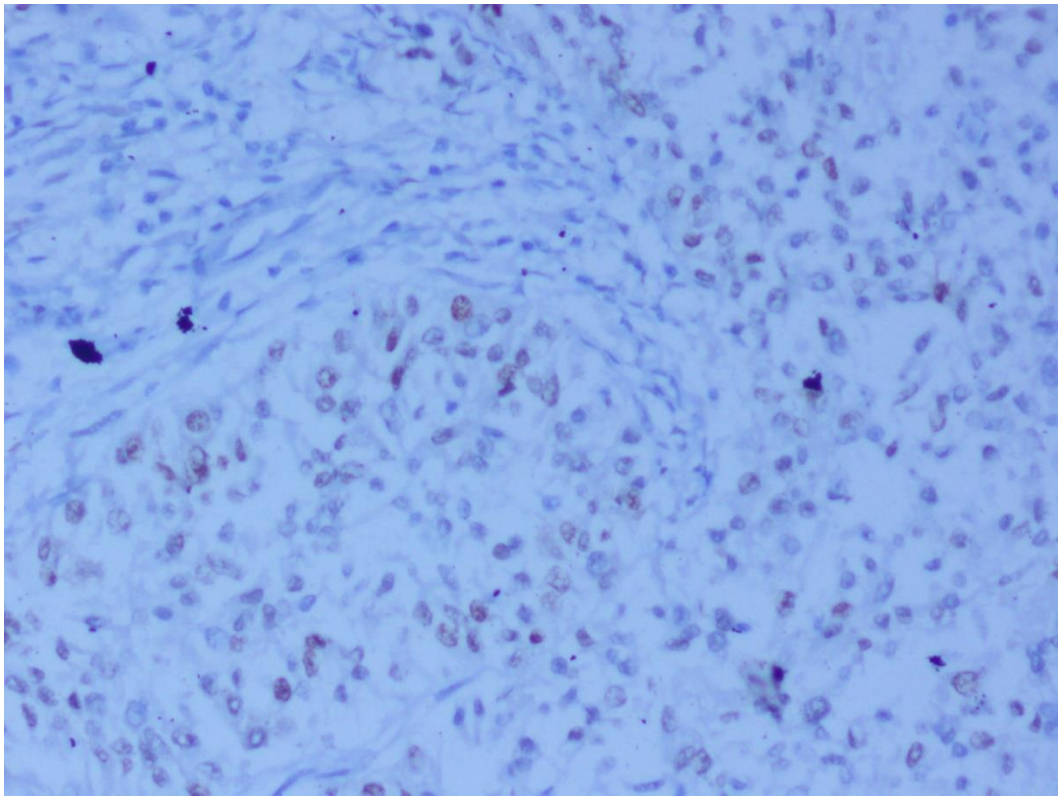

Figure 48: Poorly differentiated (Grade 3) endometrioid carcinoma with absent and weak PR expression (x200)

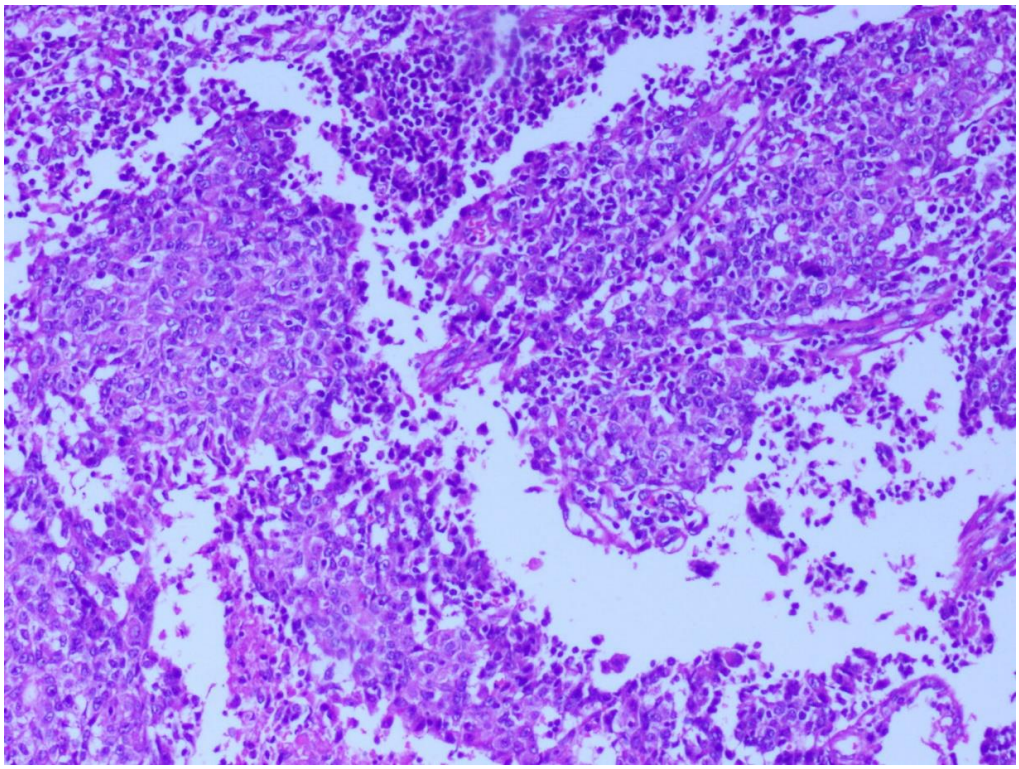

Figure 49: Serous carcinoma (H&E x100)

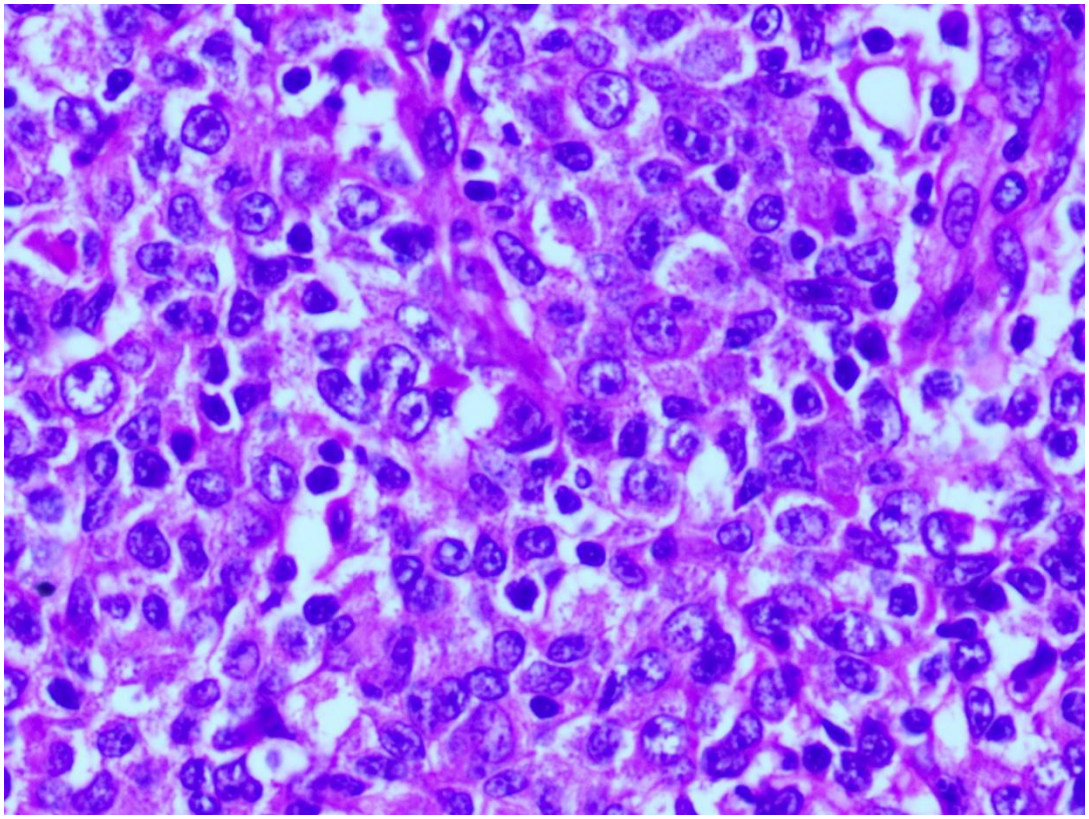

Figure 50: Serous carcinoma (H&E x400)

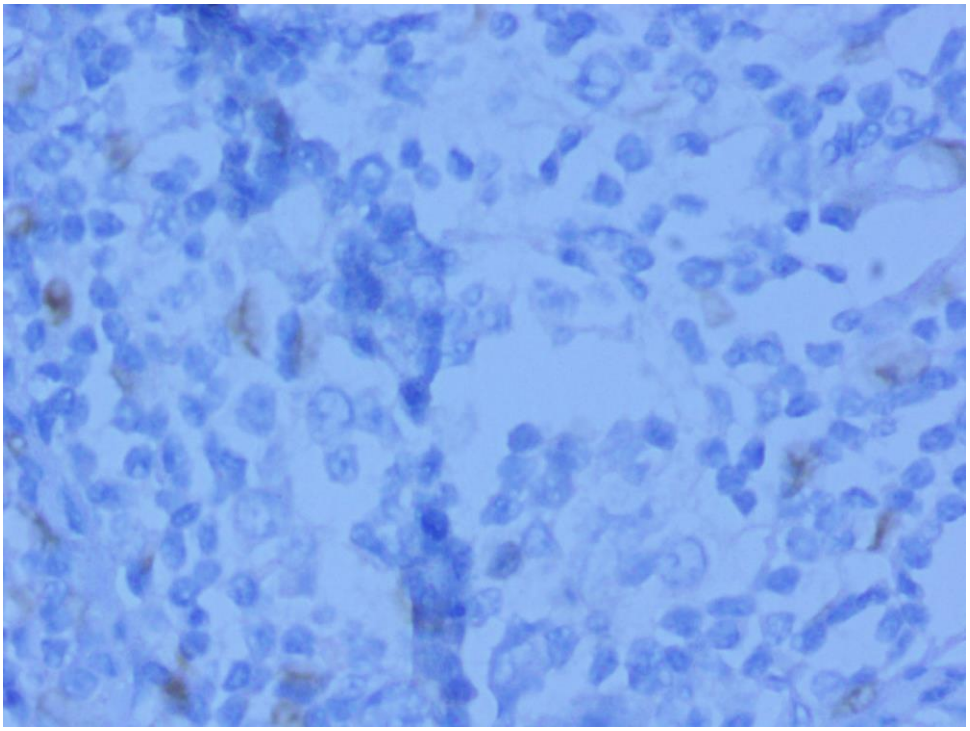

Figure 51: Serous carcinoma with absent ER expression (x400)

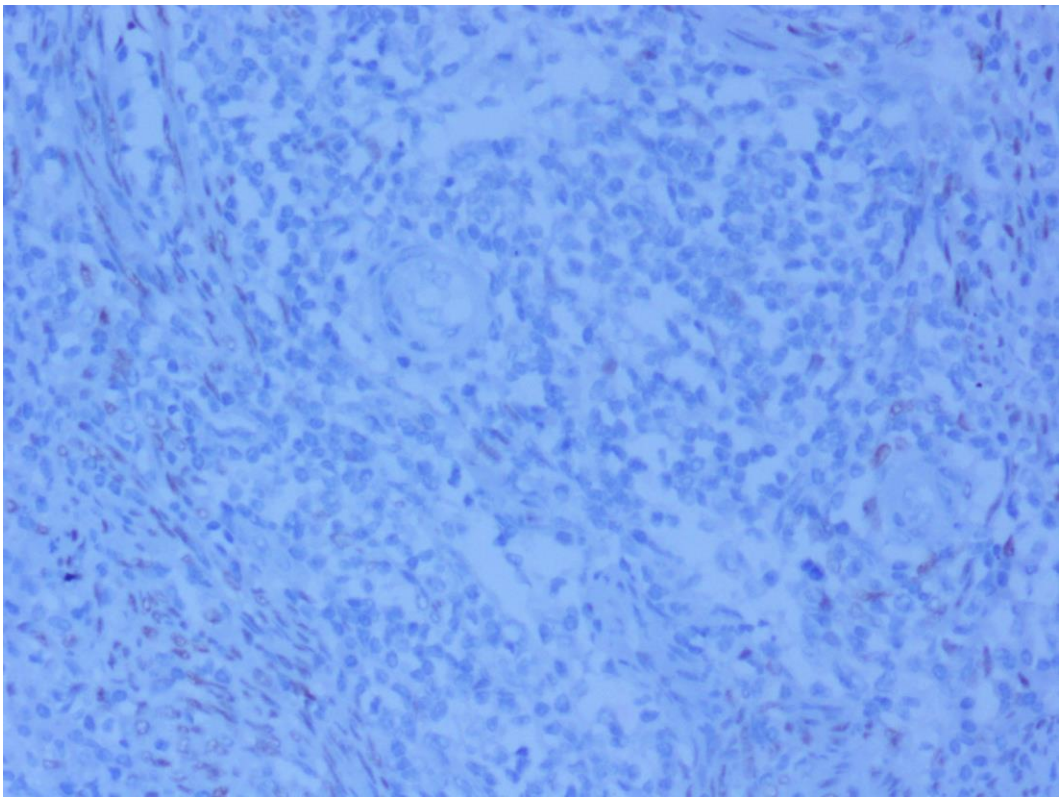

Figure 52: Serous carcinoma with absent PR expression (x200)

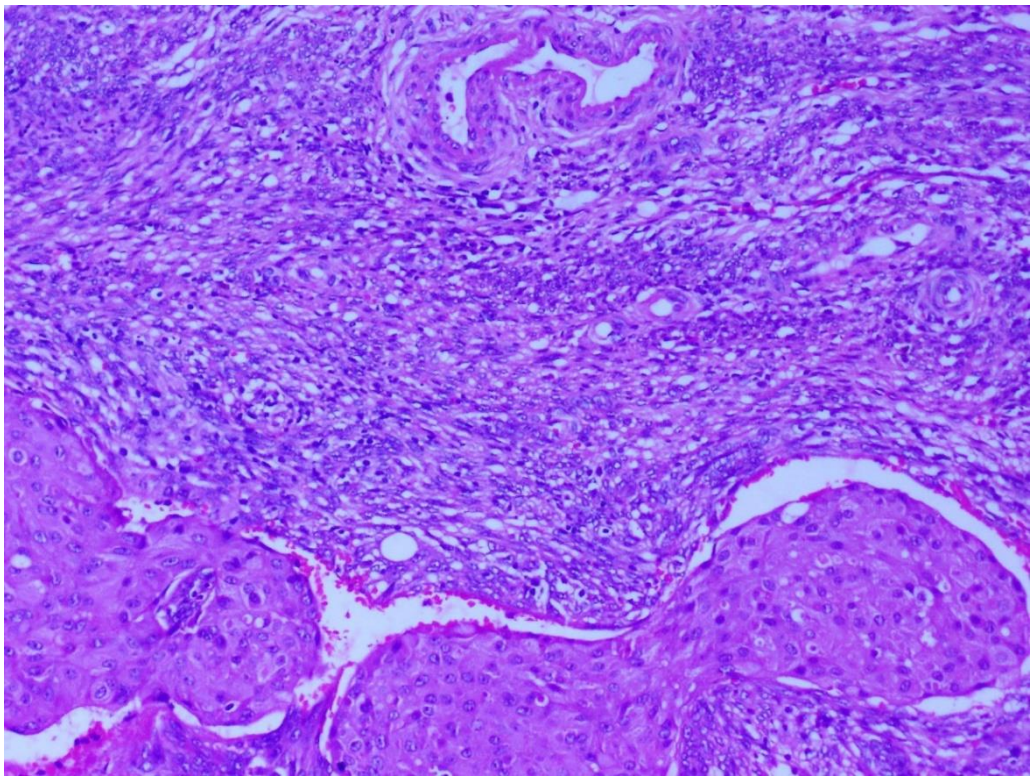

Figure 53: Carcinosarcoma (H&E x100)

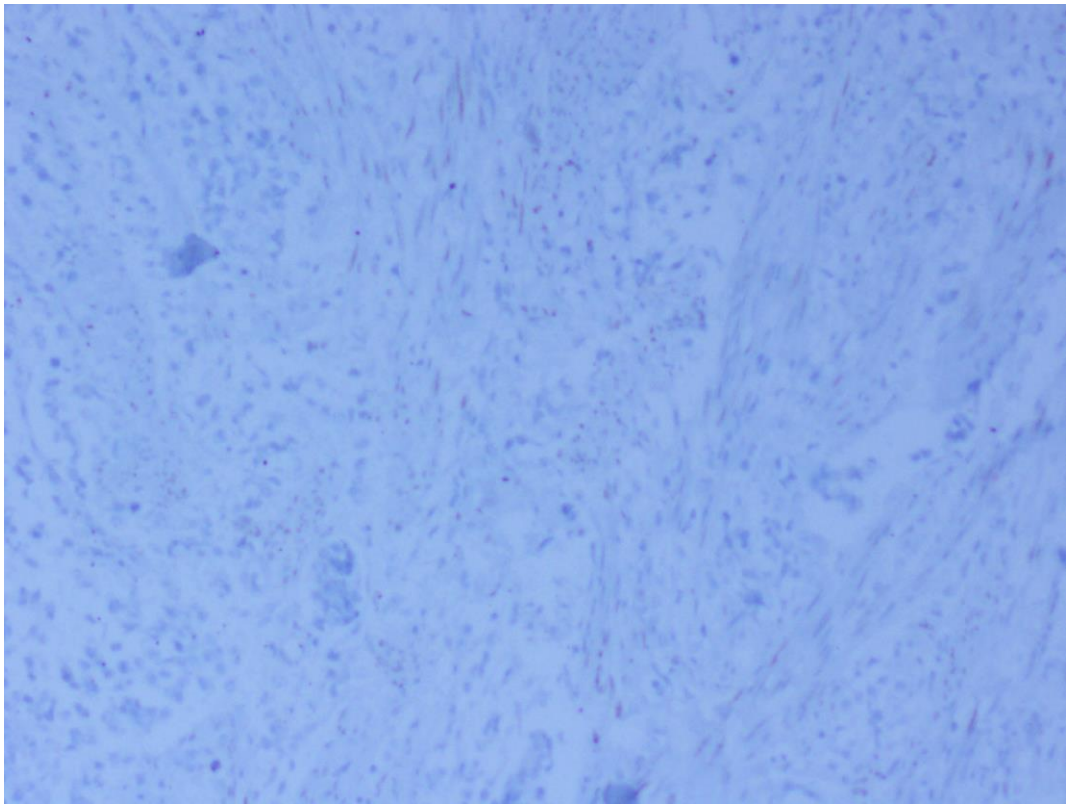

Figure 54: Carcinosarcoma with absent ER expression (x100)

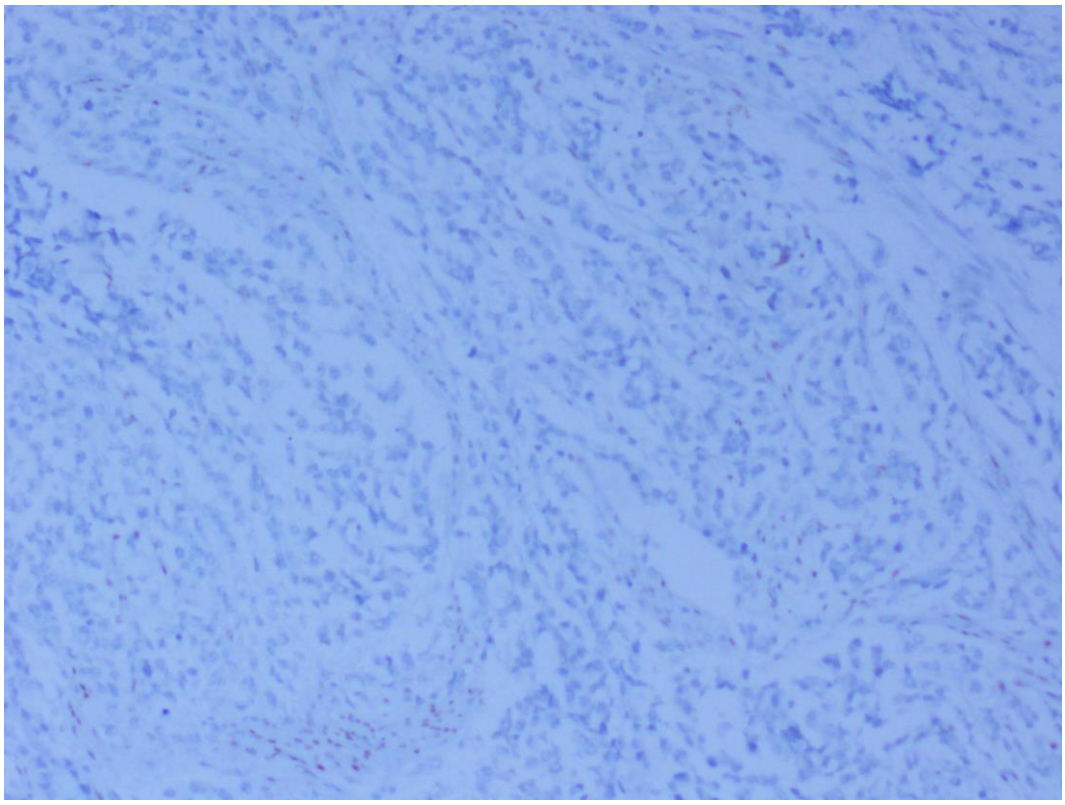

Figure 55: Carcinosarcoma with absent PR expression (x100)